# EFFECTS OF AN INTRODUCED CRAYFISH ON NATIVE ARIZONA FISHES

Jeanette Carpenter

and

Carole McIvor

Arizona Cooperative Fish and Wildlife Research Unit
Bio Sciences East Room 104, University of Arizona, Tucson, AZ 85721

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Effects of Exotic Crayfishes on Native Fish

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## EXECUTIVE SUMMARY

The virile crayfish (*Orconectes virilis*), a species alien to Arizona waters, now occurs in numerous streams throughout the state. We report results of research designed to examine impacts of this crayfish on several native fish species of Arizona streams. We conducted field experiments in two streams that represent very different examples of Arizona waters: Sabino Creek, a canyon-bound, desert stream on the outskirts of Tucson; and an unnamed tributary of the East Fork of the Black River, in a high-elevation meadow in the White Mountains. We also conducted laboratory experiments to examine competitive and predatory interactions between crayfish and fish.

Sabino Creek is a low-altitude desert stream and is home to only one species of native fish, the Gila chub (*Gila intermedia*), which is a candidate for listing under the Endangered Species Act. Below Bridge 9 in Sabino Canyon Recreational Area, Gila chub exist with both *O. virilis* and the piscivorous green sunfish (*Lepomis cyanellus*), another introduced species. Above Bridge 9 where we conducted our experiment, there are only Gila chub and crayfish. Sabino Creek experiences seasonal flash-flooding in winter and late summer, and high temperatures and drydown in early summer. During drydown, many reaches dry up completely, and the stream becomes a series of isolated pools with high densities of fish and crayfish. Resources likely become limiting, and the probability of interspecific competition between Gila chub and crayfish for scarce resources increases.

In 1996 we intensively sampled six isolated pools and determined that crayfish densities ranged from 3-12 individuals/$m^2$ and Gila chub ranged from 5-10 fish/$m^2$. We hypothesized that: (1) crayfish would compete with Gila chub for food since both species consume primarily algae and invertebrates; and (2) that such interspecific competition would be expressed as decreased growth rates of Gila chub where crayfish occur in high densities. To test this hypothesis, we conducted a field experiment where we manipulated crayfish densities in isolated pools from May to July 1996. Six pools were used in the experiment: we randomly assigned pools to undergo either a "low crayfish density" or "high crayfish density" treatment. All captured crayfish were removed from low-density pools; crayfish in high-density pools were marked to identify their pool location, and measured to estimate change in crayfish biomass and density during the experiment. Fish were marked by pool location and by 10 mm size-class. Over the course of the experiment, crayfish numbers decreased somewhat in high-density pools, but numbers in low-density pools were always significantly lower. At the end of the experiment, mean crayfish biomass ($\pm$ SE) remained significantly higher in high-density pools (69.9 $\pm$ 14.9 g/$m^2$) than in low-density pools (8.3 $\pm$ 5.2 g/$m^2$). Pools decreased in surface area an

average of 40% over a four-week period. There was little change in the weights of either crayfish or Gila chub. Over the course of the experiment, Gila chub declined slightly in size and weight; however, there was no statistically significant difference in weight loss as a function of crayfish density (ANOVA: $F_{1,4}=0.10$, $P=0.768$). Regardless of treatment, mean condition of Gila chub ($\pm$ SE) declined over time from 1.04 ($\pm$0.02) to 0.95 ($\pm$0.02) (ANOVA: $F_{1,4}=15.76$, $P=0.017$). Mean weight of crayfish in high-density pools did not change appreciably over the course of the experiment (ANOVA: $F_{1,2}=2.77$, $P=0.096$). We cannot reject the null hypothesis that crayfish have no effect on growth of Gila chub through competitive interactions. Possible explanations for failure to find a competitive effect include the following: dietary resource overlap between the two species is sufficiently low that interspecific competition is minimal; Gila chub respond to decreasing stream flow and associated isolation and shrinkage of pools by lowering their metabolic rate such that little or no growth occurs during these physiologically stressful times; crayfish negatively affect Gila chub growth at very low population levels, thus making it difficult to distinguish an effect of low vs. high density treatments; and our experiment had insufficient power to detect treatment effects.

We conducted a similar field experiment during fall 1996 to examine effects of *O. virilis* on benthic macroinvertebrates, the aquatic plant *Ranunculus aquatilis* and associated invertebrates, and three native fishes in a small unnamed stream at Three Forks in the White Mountains, Arizona. This small stream meanders through a grassy alpine meadow at 2,506 m. Because *O. virilis* is polytrophic, it can potentially compete for forage with all three native fish species occurring in the stream: speckled dace (*Rhinichthys osculus*), Sonora sucker (*Catostomus insignis*), and desert sucker (*C. clarkii*). We chose eight stream sections that appeared similar, each section containing both a riffle and a pool. These experimental sections were separated with plastic-coated wire-mesh fencing (termed weirs) to restrict crayfish and fish movement between treatment sections. We randomly assigned the eight sites to two treatments: low-density and high-density crayfish sites. In low-density sites, we removed as many crayfish as possible. In high-density sites, we re-introduced crayfish at a mean density of 1.7 individuals/m$^2$. *Ranunculus aquatilis* biomass was reduced in high-density sites compared to low-density sites, but the significance was marginal (ANOVA: $F_{1,6}=4.67$, $P=0.074$). Molluscs $>10$ mm were entirely absent from *R. aquatilis* samples in high-density sites. There were no other differences among other invertebrates associated with the aquatic plant. Benthic invertebrate samples showed no significant differences in invertebrate abundance or insect diversity over the course of the experiment. There was no treatment effect as measured by the relative change in biomass and condition factor among individually marked fish of any of the three fish species. Speckled dace showed an increase in biomass and condition factor at all sites, whereas suckers declined in weight

and condition in at least one site. Based on fish response, we could not reject the null hypothesis that crayfish do not compete with these native fish for forage at Three Forks.

Thus we obtained a null result in terms of fish response to different crayfish densities in both Sabino Creek and at Three Forks. Therefore, we cannot reject the null hypothesis that crayfish do not compete for food with these four species of native fish. There are several possible explanations for our results: (1) crayfish do no compete with Gila chub, speckled dace, desert sucker, or Sonora sucker for food resources (where such hypothesized competition is assessed by growth rates or condition factor of fishes); (2) extraneous (i.e., uncontrolled) variables such as environmental differences between replicate pools, and uncontrolled or undetected movements of study animals (in the case of Three Forks) obscured the effects of the crayfish density treatment; (3) the experiments were conducted for an insufficient amount of time, or were conducted at inappropriate times to detect competition; (4) crayfish negatively affect growth of these fishes at very low population densities, making it difficult to distinguish an effect of low vs. high density treatments; or (5) the experiments as designed lacked sufficient power to detect treatment effects. Crayfish, do, however, reduce benthic insect diversity, *R. aquatilis* biomass, and larger molluscs associated with *R. aquatilis*.

We also conducted laboratory experiments to determine if *O. virilis* compete for shelter with three native Arizona fishes: Gila chub (*Gila intermedia*), desert sucker (*Catostomus clarki*), and speckled dace (*Rhinichthys osculus*). Additionally, we ran experiments to determine if crayfish prey directly on Gila chub and desert sucker. For the competition experiments, we used a crayfish that was of equal or smaller size than the three native fish. In these competition experiments, we used green sunfish (*Lepomis cyanellus*) as a predator to elicit a stronger response from both crayfish and native fish in seeking shelter. Crayfish displaced native fish from shelter and attacked them several times. None of the native fish attacked the crayfish, and out of 19 trials, only one native fish (a desert sucker) displaced a crayfish. Although native fish sought cover during control trials and when green sunfish were visible through a clear partition, they never used shelter for refuge when the partition was removed.

We evaluated vulnerability of Gila chub and desert sucker to predation by large crayfish (>3.5 cm carapace length). Crayfish preyed upon both fish species; however crayfish preyed more heavily upon desert suckers than on Gila chub. It is likely that desert suckers were more vulnerable because they used primarily the lower portion of the water column, whereas Gila chub used the entire water column. Neither native fish species altered their use of the water column in the presence of crayfish. This lack of a behavioral response to a predator demonstrates "naivety" and likely derives from the lack of a common evolutionary history.

In conclusion, our field experiments yielded no evidence that crayfish adversely affect either growth rates or condition factor of four species of native fishes via competition for food, despite the fact that crayfishes at Three Forks significantly reduced biomass of *Ranunculus aquatilis* and some invertebrates in experimental stream treatment sections. Behaviors observed in a laboratory setting, however, indicate that crayfish do prey upon native fishes and successfully compete with fishes for refuge from predators. We do not know to what degree the behaviors observed in the laboratory also occur under field conditions. However, predation under field conditions seems likely, especially when drought reduces streams to isolated pools and increases densities of both fish and crayfish, thereby enhancing the probability of interspecific interactions. Whereas such a drydown is probably a rare event in high altitude streams such as Three Forks, drydown is a very common condition in desert streams similar to Sabino Creek. Native Arizona fishes did not evolve with a crustacean predator and they may lack appropriate behaviors for recognizing, and escaping from, such a predator.

Further research is clearly needed to determine if our laboratory results apply to field situations. Additionally, replication of our field studies is highly recommended with the following modifications: more replicate pools or treatment sections within a given stream; and fish individually marked, possibly with fingerling tags. Because of the difficulty of controlling movement of marked organisms of small size between treatment sections in an experiment, streams with isolated replicate pools provide a potentially powerful experimental setting for addressing questions of competition and predation. Replicated microcosms would also provide a useful model for pursuing these questions.

# RESULTS OF A FIELD EXPERIMENT TO EXAMINE THE EFFECTS OF AN INTRODUCED CRAYFISH (*ORCONECTES VIRILIS*) ON GILA CHUB (*GILA INTERMEDIA*) IN SABINO CANYON, ARIZONA

Jeanette Carpenter

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Carole McIvor

Arizona Cooperative Fish and Wildlife Research Unit
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# ABSTRACT

The virile crayfish (*Orconectes virilis*), a species alien to Arizona waters, occurs in Sabino Creek, Pima County, Arizona. We examined effects of crayfish on Gila chub (*Gila intermedia*), the only native fish now occurring in Sabino Creek, by conducting a field experiment where we manipulated crayfish densities in isolated pools from May to July 1996. As pools shrink during early-summer drought, resources likely become limiting, and the probability of interspecific competition between Gila chub and crayfish for scarce resources increases. We hypothesized that crayfish would negatively affect Gila chub growth (measured as weight gain) during this period. Six pools were used in the experiment: we randomly assigned pools to undergo either a "low crayfish density" or "high crayfish density" treatment. At the beginning of the study, before crayfish densities were manipulated, crayfish densities ranged from 3-12 individuals/$m^2$ and Gila chub ranged from 5-10 fish/$m^2$. All captured crayfish were removed from low-density pools; crayfish in high-density pools were marked to identify their pool location, and measured to estimate change in crayfish biomass and density during the experiment. Fish were marked by pool location and by size-class. Over the course of the experiment, crayfish numbers decreased somewhat in high-density pools, but numbers in low-density pools were always significantly lower. At the end of the experiment, mean crayfish biomass (± SE) remained significantly higher in high-density pools (69.9 ± 14.9 g/$m^2$) than in low-density pools (8.3 ± 5.2 g/$m^2$). Pools decreased in water level an average of 27 cm over a four-week period. There was little change in the weights of either crayfish or Gila chub. Over the course of the experiment, Gila chub appeared to decline slightly in size and weight; however, there was no statistically significant difference in amount of weight lost as a function of crayfish density. Regardless of treatment, mean condition of Gila chub (± SE) declined over time from 1.04 (±0.02) to 0.95 (±0.02) (ANOVA; $F_{1,4}$=15.76, $P$=0.017). Mean weight of crayfish in high-density pools did not change appreciably over the course of the experiment. We cannot reject the null hypothesis that crayfish have no effect on growth of Gila chub through competitive interactions. Possible explanations for failure to find a competitive effect include the following: dietary resource overlap between the two species is sufficiently low that interspecific competiton is minimal; Gila chub respond to decreasing stream flow and associated isolation and shrinkage of pools by lowering their metabolic rate such that little or no growth occurs during these physiologically stressful times; crayfish negatively affect Gila chub growth

at very low population levels, thus making it difficult to distinguish an effect of low vs. high density treatments; and our experiment had insufficient power to detect treatment effects.

## INTRODUCTION

The introduction of non-native organisms to aquatic ecosystems is considered a major factor in the decline of native fish (Moyle et al. 1986; Douglas et al. 1994). A recent review of causes for placing species on the federal endangered species list rated interactions with non-native species as one of the most common factors (Czech and Krausman 1997). Crayfish, which have been introduced into freshwater systems throughout the United States, have the potential to cause a variety of ecological changes when introduced into new systems. They may become pest species because they are opportunistic omnivores and can impact aquatic systems at several trophic levels (Lorman and Magnuson 1978; Hobbs et al. 1989), and they quickly outgrow predation by most fishes due to their large size and aggressive behavior (Lodge et al. 1985). Non-native crayfish can reduce abundance of macrophytes (Dean 1969; Lodge and Lorman 1987; Chambers et al. 1990; Lodge et al. 1994), native crayfish (Lodge et al. 1985; Momot et al. 1978; Hill and Lodge 1994), and other macroinvertebrates (Hanson et al. 1990; Charlebois and Lamberti 1996; Hoekstra 1998). Research on interactions between non-native crayfish and fish has focused primarily on how fish predation influences replacement of native crayfish by non-native crayfish (e.g., Mather and Stein 1993; Garvey et al. 1994).

As early as 1959, non-native crayfish were blamed for declines or displacement of native crayfish in California (Riegel 1959), Oregon (Bouchard 1978) and in various states east of the Continental Divide (Bouchard 1976). The most famous and intensely-researched example is the displacement of native crayfish in Wisconsin by *Orconectes rusticus* (e.g., Lodge et al. 1985; Garvey et al. 1994; Hill and Lodge 1994). Recently, several biologists recognized the potential for non-native crayfish to cause negative impacts on aquatic systems in the western U.S. (Johnson 1986; Hepworth and Duffield 1987; Hubert 1988; Fernandez and Rosen 1996; Hoekstra 1998).

Only a few studies have quantified--or even discussed--the effect of crayfish on nongame native fishes. Courtois and Tippets (1979) noted abundant numbers of Owens pupfish *(Cyprinodon radiosus)* in Warm Springs, California in 1977. However, in 1979 they determined that the pupfish population was extirpated and that *Procambarus clarkii* had became abundant at the site.

Unfortunately, information determining if crayfish caused the disappearance of the pupfish was not available. Cave Creek near Phoenix, Arizona supported Gila topminnow (*Poeciliopsis occidentalis occidentalis*) as well as other fishes endemic to the Gila River. Since the invasion of *Orconectes virilis*, the rare fish disappeared (S. Stefferud, USFWS, personal communication); however, no cause and effect relationship is known. Two papers noted crayfish feeding upon non-game fishes but do not provide quantitative data on predation rates (Dean 1969; Rahel and Stein 1988). Guan and Wiles (1997) reported benthic fish mortalities of 11-17% when kept in artificial streams with crayfish, vs. mortalities of <1% in control pools.

Crayfish are not native to Arizona (Hobbs Jr. 1988). However, at least two species are now found in its streams: *Procambarus clarkii* and *Orconectes virilis* (Hobbs Jr. 1972). Threatened and endangered fishes that inhabit small streams and springs in Arizona may be more vulnerable to crayfish introductions than species that evolved with crayfish. Predatory control of crayfish may be minimal in these streams because native fish are generally small and crayfish quickly outgrow predation by even large fish (Stein 1977). Possible impacts to endangered, small fishes from non-native crayfish include competition between crayfish and small benthic fishes for cover (Rahel and Stein 1988; McNeely et al. 1990) and food (Miller et al. 1992); direct predation on fish by crayfish (Hobbs III 1993); and reduction in macrophytes that native fish may require for cover, nursery habitat, and as a source of macroinvertebrates (Lodge et al. 1985; Chambers et al. 1990).

Research that focuses on the impact of non-native crayfish on native fishes in Arizona is especially timely, considering: 1) the importance of restoring habitats for native fish in the Southwest; 2) the regional decline in native fish populations over the last century (Williams et al. 1989; Minckley and Douglas 1991); and 3) the lack of basic ecological information on these fishes.

In this study, we conducted a field experiment to determine if crayfish affect growth rates and condition of Gila chub. We manipulated crayfish numbers in several pools within a stream section where Gila chub is the only fish species, and measured weight and length of different size classes of Gila chub to determine if there was a crayfish effect. We also collected information on natural densities of Gila chub and crayfish during early summer, and measured crayfish biomass, water chemistry, and physical habitat.

Gila chub

Gila chub is endemic to the Gila River Basin of Arizona, New Mexico, and Sonora, Mexico (Minckley 1985). It is considered to be extirpated from New Mexico (Sublette et al. 1990) and only

occurs in 24 isolated streams or cienegas in Arizona and Mexico (Weedman et al. 1996). The main threats to Gila chub are habitat loss and non-native predaceous and competitive fishes (Weedman et al. 1996). Gila chub is a candidate for listing under the Endangered Species Act (1997 Notice of Review, 9/19/97).

In Sabino Canyon, Gila chubs prefer low velocity pools and areas > 0.3 m deep during all seasons (Dudley 1995) However, in winter they prefer areas close to cover, such as interstitial spaces between boulders. They feed primarily on terrestrial and aquatic insects and filamentous algae (Griffith and Tiersch 1989). *Orconectes virilis* is also omnivorous, and feeds on aquatic invertebrates and plants (Chambers et al. 1990; Hanson et al. 1990).

Study Area

This field experiment was conducted within the Sabino Canyon Recreation Area of the Coronado National Forest in Pima County, Arizona. Sabino Creek is a tributary of Tanque Verde Wash and Rillito Creek in the Santa Cruz River drainage, which is within the Gila River basin. Green sunfish (*Lepomis cyanellus*) occur in the lower portion of the canyon below bridge 9 (Dudley 1995). We conducted our work in a 0.5-km section upstream of Bridge 9 (Figure 1), where Gila chub is the only fish species present.

## MATERIALS AND METHODS

We used isolated pools as experimental replicates to examine differences in growth rates of Gila chub when crayfish are present in high densities compared with low densities. Our research hypothesis was that crayfish reduce growth rate or condition of Gila chub by competitive interactions.

We located six pools that contained crayfish and Gila chub, and collected physical habitat and water chemistry data to evaluate similarity among pools. Physical habitat was measured by setting up five equidistant transects across a pool, and determining depth and dominant substrate every 0.5 m along the transects. Surface area was determined by averaging widths of the five transects and multiplying by total length of the pool. Permanent marks placed on an overhanging boulder in each pool allowed us to measure change in water level over time. The physical habitat data were collected on 30 May 1996. Water chemistry was measured on 4 and 25 June 1996 with a Hydrolab H-20.

Pools were randomly assigned to be either low-density pools, in which we removed as many crayfish as possible, or high-density pools, in which we only estimated the crayfish population or in some cases supplemented it so that densities across pools were similar. We intensively surveyed all six pools for crayfish and fish in as short a time period as possible, so that the "treatment" period was clearly delineated. We primarily used metal minnow traps with enlarged openings to capture crayfish. We used up to 20 traps in each pool; half were baited with canned dog food to attract crayfish, and half were set up without bait to attract only Gila chub. Traps were usually set in early evening and emptied in the morning. During the day we set traps and electrofished for Gila chub, and at night we hand-caught crayfish using flashlights and small dip nets. In low-density pools, we attempted to remove all crayfish. We conducted a depletion estimate of the fish and crayfish populations by making repeated trapping and electrofishing passes, and holding captured crayfish and fish in separate cages until the end of the last removal pass (White et al. 1982). We measured carapace length (CL) and weight of all crayfish caught in high-density pools, and marked them with pleural clips to identify their pool origin, in case they moved between pools. During the course of the experiment, we intermittently trapped for crayfish in low-density pools to ensure there was a significant depression if not depletion of crayfish in these pools, since some crayfish might travel overland into adjacent pools.

Fish were anesthetized with tricaine methane sulfonate before measuring and marking. We marked as many fish > 4 cm as possible in each pool with fluorescent elastomer. Marks differed for each length class (1-cm size class) and each pool. We put a maximum number of two marks on each fish, and kept fish in a 0.5% salt solution until release to reduce mortality, as recommended by Haines and Modde (1996). By marking fish at the beginning of the study and then recapturing them at the end of the study period, we were able to measure changes in mean weight and length of that size class of fish and also determine if fish moved between pools. We recorded total length (TL) to the nearest mm of all marked fish. To obtain an accurate fish weight, we weighed each fish three times to the nearest 0.01 g and then took a mean of these weights. We also recorded appearance (e.g., fin condition) of each fish. Numbers of each size-class per pool were estimated based on our catches. We accounted for difference in surface areas between pools and over time by comparing fish and crayfish populations in terms of density (number of fish or biomass/$m^2$) instead of actual abundances.

We ran the field experiment for as long as possible before the summer monsoons began and reconnected the pools. At the completion of the experiment, crayfish and fish were captured, weighed, and measured to determine treatment effects.

Statistical Analyses

This experiment is a randomized factorial design. To compare fish growth among treatments, we used a multiple-factor ANOVA in which effects were treatment (low- or high-density crayfish), pools, time (pre- and post-experiment), and size-class of fish (1-cm intervals). Fish length, weight, and Fulton's condition factor, or K (100,000 X g/mm$^3$) were the response variables in three separate ANOVA's. We could not assign a pre- and post-treatment weight, length, or condition factor to a given fish. Therefore, individual fish measured before and after the experiment were considered independent during preliminary analysis. Each response variable was first tested for pool effects, which were nested within treatments. A significant effect for pools nested within crayfish treatment indicated that there was a pool effect; if this occurred we collapsed the fish data and compared response variables at the level of mean response for each size class within a pool. Thus pools rather than individuals within pools became the units of replication. We used ANOVA to compare mean weight of marked crayfish in high-density pools pre- and post-experiment; main effects were pools, time, and their interaction. We did not compare weights of crayfish in low-density pools over time since all crayfish captured in these pools at the beginning of the experiment were removed to create the low-density treatment. An $\alpha$-level of 0.05 was used to determine significance of statistical tests.

## RESULTS

The field experiment ran for approximately four weeks in each pool (Table 1). However, actual dates that pools were used in the experiment were staggered due to the time necessary to process fish and crayfish, and also because we needed to wait for water levels to drop in some pools to ensure their isolation.

Habitat and water chemistry data at the beginning of experiment

The primary substrates in pools were bedrock, boulder, gravel and sand (Table 2). Surface areas of pools decreased dramatically over time: on average, pools shrank by 40% over the course of the experiment. The amount of pool shrinkage (measured as a percent of beginning surface area) not differ significantly between pools in the two treatment groups ($t \leq 1.07$, df=4, $P \leq 0.35$).

Table 1. Schedule of field experiment in Sabino Creek.

| Pool | Crayfish treatment | Beginning date | End date | Total number of days experiment ran |
|---|---|---|---|---|
| 1 | High-density | 17 May 96 | 14 June 96 | 28 |
| 2 | Low-density | 16 May 96 | 12 June 96 | 27 |
| 3 | High-density | 23 May 96 | 19 June 96 | 27 |
| 4 | Low-density | 25 May 96 | 21 June 96 | 27 |
| 5 | Low-density | 01 June 96 | 27 June 96 | 26 |
| 6 | High-density | 11 June 96 | 09 July 96 | 28 |

Table 2. Physical habitat of experimental pools. Mean depth and proportion of substrates are from surveys at beginning of experiment. Data on surface areas correspond to dates shown in Table 1.

| Pool | Beginning Surface Area $m^2$ | End Surface Area $m^2$ | Mean Depth m | Relative abundance of substrates (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Bedrock | Boulder > 256 mm | Cobble 64-256 mm | Gravel/ Sand < 64 mm | Wood/ Leaf |
| 1 | 27.12 | 17.20 | 0.270 | 25 | 23 | 4 | 48 | 0 |
| 2 | 47.04 | 19.83 | 0.338 | 15 | 26 | 8 | 49 | 1 |
| 3 | 54.89 | 44.95 | 0.271 | 22 | 32 | 12 | 35 | 0 |
| 4 | 28.79 | 23.99 | 0.316 | 0 | 26 | 18 | 52 | 4 |
| 5 | 89.64 | 24.81 | 0.213 | 10 | 7 | 0 | 78 | 6 |
| 6 | 16.03 | 10.34 | 0.418 | 55 | 0 | 32 | 14 | 0 |

Temperature was relatively similar among pools (Table 3). Pool 4 had a relatively low pH whereas Pool 6 had a relatively high pH. Pool 4 also had lower than average dissolved oxygen levels. Low conductivities may indicate subsurface waterflow into a pool: groundwater typically has less dissolved solids than surface water. Low conductivities could also indicate a slower evaporation rate, since dissolved solids will concentrate with increasing evaporation. Therefore the data in Table 3 suggest that Pools 2, 3, and 4 might be less likely to dry up than the other pools. In fact, water levels in Pools 4 and 6 declined the least: they sustained a 4 and 12 cm decrease in water level, respectively, whereas the other pools dropped 27-50 cm in a four-week period. There was no significant difference in pH, temperature, or conductivity between pools in the two treatment groups (t-test; $P<0.1$). However, there was a significant difference between treatments for dissolved oxygen (DO) levels collected on 25 June. Low-density pools had significantly lower DO levels than high-

Table 3. Water chemistry of experimental pools. Data were collected between 10:00 A.M. and 1:00 P.M. on 4 June and 7:00-8:00 A.M. on 25 June.

| Pool | Crayfish treatment | Date | Temperature (°C) | pH | Conductivity (µS/cm) | Dissolved oxygen (% saturation) |
|---|---|---|---|---|---|---|
| 1 | High-density | 4 June | 24.9 | 7.8 | 246 | 127 |
| | | 25 June | 21.6 | 7.4 | 369 | 53 |
| 2 | Low-density | 4 June | 22.2 | 8.0 | 183 | 102 |
| | | 25 June | 20.1 | 7.2 | 261 | 23 |
| 3 | High-density | 4 June | 22.5 | 7.9 | 137 | 101 |
| | | 25 June | 21.8 | 7.0 | 258 | 49 |
| 4 | Low-density | 4 June | 23.4 | 6.2 | 149 | 40 |
| | | 25 June | 23.4 | 6.1 | 150 | 11 |
| 5 | Low-density | 4 June | 25.3 | 7.8 | 222 | 94 |
| | | 25 June | 19.9 | 7.2 | 256 | 31 |
| 6 | High-density | 4 June | 25.9 | 9.6 | 223 | 139 |
| | | 25 June | 23.5 | 9.0 | 287 | 65 |

density pools ($t$=-4.51, df=4, $P$=0.011); the low-density pools averaged 22% DO (± 5.8 SE) whereas the high-density pools averaged 56% DO (± 4.8 SE). There was no difference between treatments for the higher DO levels collected on 4 June; in addition, higher DO levels collected on two other dates with a YSI meter were not significantly different between treatments (data not included).

Crayfish Densities

We caught a total of 1,396 crayfish at the beginning of the experiment. Crayfish density originally varied among pools; it ranged from 1.6 individuals/$m^2$ in Pool 6 to 10.9 individuals/$m^2$ in Pool 4 (Table 4). Biomass per unit area also varied. Our ability to deplete crayfish numbers was not affected by original densities or by total numbers of crayfish. In low-density pools, we reduced the crayfish population by 87-100%. We opportunistically removed smaller crayfish (those with CL<25 mm) from low-density pools, but we did not attempt a complete removal due to the difficulty in efficiently capturing these smaller crayfish. Not all pools contained smaller crayfish; however their biomass has been added to overall estimates of crayfish biomass in each pool (Table 4).

Crayfish densities remained higher in high-density pools than in low-density pools (Table 5), in terms of both numbers ($t$=-3.89, df=4, $P$=0.018) and biomass ($t$=-4.54, df=4, $P$=0.011). During the experiment, crayfish numbers and biomass decreased in all pools. Pools 1, 2, 3, and 5 were adjacent to each other and we expected crayfish to move out of pools as they dried up. Yet out of 676 marked crayfish, we found only one crayfish that had moved from one pool to another (from Pool 3 to Pool 5). There was a dramatic drop in crayfish numbers in Pool 6 (Table 4). However, despite varying decreases in numbers of crayfish among pools over the course of the experiment, densities remained significantly higher in high-density pools than in low-density pools (Table 5; $t$=3.59, df=4, $P$=0.023).

Fish Densities

At the beginning of the experiment, we caught, marked, and measured 1,315 fish. The total number of fish we observed in pools was somewhat higher; we counted but did not always measure fish that were less than 40 mm or greater than 100 mm. Pool 5 had many fish of 5-6 cm that we did not weigh or mark; we only marked 131 of the 274 5-6 cm fish that we caught. We have included unmarked fish in our density estimates for each pool (Table 6).

Table 4. Crayfish densities pre- and post-experiment. Numbers are based on total numbers of crayfish > 25 cm caught; smaller crayfish are included in the biomass estimates. Densities are based on surface areas shown in Table 2. * = Original numbers found in Pool 6 before we added more crayfish.

| Pool | Crayfish Treatment | Total numbers of crayfish > 25 cm | | Total biomass of crayfish (g) | | Density of crayfish # / $m^2$ | | Crayfish biomass per unit area g / $m^2$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| 1 | High-density | 267 | 131 | 4425.6 | 2109.5 | 9.85 | 7.62 | 163.2 | 122.7 |
| 2 | Low-density | 137 | 0 | 1658.5 | 0.0 | 2.91 | 0.00 | 35.3 | 0.0 |
| 3 | High-density | 291 | 220 | 4058.7 | 3858.3 | 5.30 | 4.90 | 73.9 | 85.8 |
| 4 | Low-density | 314 | 23 | 4309.9 | 500.8 | 10.91 | 0.96 | 149.7 | 20.9 |
| 5 | Low-density | 269 | 40 | 4660.1 | 647.2 | 3.00 | 1.61 | 52.0 | 26.1 |
| 6 | High-density | 28*<br>118 | 41 | 180.4*<br>2154.6 | 742.2 | 1.6*<br>7.3 | 3.97 | 10.3*<br>134.2 | 71.8 |

Table 5. Comparison of crayfish densities at the end of the experiment.

| Crayfish Treatment | Crayfish Numbers # / $m^2$ ( x ± SE) | Crayfish Biomass g / $m^2$ ( x ± SE) | Decrease in Crayfish (# of crayfish at beg - # of crayfish at end)/ # of crayfish at beg. % |
|---|---|---|---|
| High-density | 5.5 ± 1.10 | 93.4 ± 15.17 | 0.47 ± 0.12 |
| Low-density | 0.9 ± 0.47 | 15.7 ± 7.97 | 0.93 ± 0.04 |

Densities of fish ranged from 3.9 to 9.5 individuals/$m^2$ at the beginning of the experiment (Table 6); at the end fish densities had increased as pool size decreased in 2 of 6 pools. At the end of the experiment, fish numbers and densities across pools were fairly close except for Pool 1, which had a 100% increase in fish numbers from beginning to end. Seventy percent of these new fish were <60 mm; these fish must have been small enough at the beginning of the experiment to escape capture by passing through the mesh openings of the minnow traps, i.e., <40 mm.

We recaptured 78-91% of all marked fish (Table 7). There was no difference in recapture rates by treatment ($t$=0.187, df=4, $P$=0.861).

Field Experiment

There was a significant effect for pools nested within crayfish treatment for our three response variables: total length, weight, and condition factor of Gila chub (Table 8). In other words, response variables for pools within a crayfish treatment differed significantly among each other. Because the effect of pools within treatments could not be ignored, the unit of analysis became the pool rather than

Table 6. Fish densities pre- and post-experiment. The numbers presented here are for all caught fish, included those that were not marked. * = Original numbers found in Pool 6 before we added more fish.

| Pool | Crayfish treatment | Total number of fish caught | | Density of fish # / $m^2$ | |
|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post |
| 1 | High-density | 214 | 444 | 9.5 | 19.7 |
| 2 | Low-density | 221 | 236 | 5.1 | 5.5 |
| 3 | High-density | 322 | 266 | 6.2 | 5.1 |
| 4 | Low-density | 137 | 134 | 4.9 | 4.8 |
| 5 | Low-density | 621 | 610 | 6.6 | 6.5 |
| 6 | High-density | 26*<br>95 | 80 | 1.5*<br>5.5 | 4.6 |

Table 7. Recapture rates of marked fish by pool and size-class.

| | | Size class of fish at beginning of experiment | | | | | | | | | | | | | | All fish | | Recapture rates for all fish |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 40-49 mm | | 50-59 mm | | 60-69 mm | | 70-79 mm | | 80-89 mm | | 90-99 mm | | >100 mm | | | | |
| Pool | Crayfish Treatment | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre/ Post |
| 1 | High-density | 3 | 3 | 98 | 90 | 24 | 22 | 51 | 43 | 20 | 18 | 3 | 2 | 12 | 9 | 211 | 187 | 0.89 |
| 2 | Low-density | 11 | 6 | 128 | 106 | 14 | 13 | 10 | 9 | 20 | 15 | 16 | 9 | 16 | 10 | 215 | 168 | 0.78 |
| 3 | High-density | 63 | 48 | 183 | 149 | 33 | 30 | 7 | 6 | 10 | 7 | 9 | 4 | 6 | 3 | 311 | 247 | 0.79 |
| 4 | Low-density | 1 | 1 | 20 | 17 | 76 | 74 | 10 | 9 | 15 | 13 | 10 | 8 | 4 | 2 | 136 | 124 | 0.91 |
| 5 | Low-density | 62 | 40 | 131 | 109 | 28 | 23 | 47 | 41 | 22 | 21 | 20 | 18 | 37 | 25 | 347 | 277 | 0.80 |
| 6 | High-density | 0 | 0 | 13 | 9 | 37 | 27 | 16 | 14 | 22 | 19 | 6 | 4 | 1 | 1 | 95 | 74 | 0.78 |

individual fish. This necessary procedure resulted in a loss of power because our sample size changed from >2000 (fish data points) to 6 (pools).

Changes in mean weight, condition factor and total length across pools and time were similar among size-classes of Gila chub regardless of crayfish treatment (Figures 2a-2c). The ANOVA's for all three response variables (weight, condition, and total length) indicated no significant effect of treatment over time by size class ($P \geq 0.33$ for Trt*Time effect; Table 8). In other words, for all size-classes of Gila chub, differences in weight, condition, or total length over time were not significantly different between crayfish treatments.

In high-density pools, mean weight of Gila chub decreased from 3.18 g (±0.17 SE) at the beginning of the experiment to 3.00 g (±0.17 SE) at the end. In low-density pools, mean weight of Gila chub decreased from 4.80 g (±0.29 SE) to 4.10 g (±0.27 SE).

There was a significant change in condition factor over time ($P$=0.017; Table 8): regardless of treatment, the condition of fish declined from an average of 1.04 (±0.02 SE) to an average of 0.95 (±0.02 SE). Thus in this experiment, crayfish densities produced no apparent changes in Gila chub growth rate or condition.

The mean weight of crayfish over all high-density pools did not change appreciably during the course of the experiment with respect to time (Figure 3). The ANOVA results for change in mean weight of crayfish revealed significant effects of pool and the time*pool interaction but not over time alone (Table 9). Thus, change in mean crayfish weight was inconsistent between pools. We compared the weight change in each pool with one-way ANOVA's using contrasts (Table 10), and determined that crayfish in Pool 1 showed a significant gain in weight over time, however Pools 3 and 6 showed no significant change in either direction.

## DISCUSSION

We examined effects of crayfish on Gila chub by manipulating crayfish densities in six isolated pools. As pools shrink during early-summer drought, resources of food and space likely become limiting. Competition is expected to be most intense when resources are limiting (Wiens 1989) and at high population densities (Matthews 1998). We hypothesized that crayfish would affect Gila chub weight gain and condition factor during this period. We were successful in maintaining either high or low treatment

Table 8. Summary of analyses of variance testing effects of crayfish treatment (low or high density), time (pre and post-experiment) and their interaction on mean weight, condition factor, and total length of Gila chub.

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| Mean weight (g) | Trt | 1 | 63.11 | 63.11 | 0.72 | 0.444 |
| | Error(Trt) | 4 | 350.28 | 87.57 | | |
| | Time | 1 | 9.30 | 9.30 | 1.25 | 0.326 |
| | Trt*Time | 1 | 0.74 | 0.74 | 0.10 | 0.768 |
| | Error(Time(Trt)) | 4 | 29.69 | 7.42 | | |
| | Class | 6 | 18,617.49 | 3,102.92 | 35.88 | 0.0001 |
| | Trt*Class | 6 | 186.97 | 31.16 | 0.36 | 0.896 |
| | Error(Class(Trt)) | 23 | 1,989.23 | 86.49 | | |
| | Time*Class | 6 | 15.10 | 2.52 | 0.36 | 0.895 |
| | Trt*Time*Class | 6 | 1.54 | 0.26 | 0.04 | 0.999 |
| | Error(Time*Class(Trt)) | 23 | 159.36 | 6.93 | | |
| Condition factor (K) | Trt | 1 | 0.05 | 0.05 | 0.21 | 0.669 |
| | Error(Trt) | 4 | 0.92 | 0.23 | | |
| | Time | 1 | 0.85 | 0.85 | 15.76 | 0.017 |
| | Trt*Time | 1 | 0.06 | 0.06 | 1.12 | 0.350 |
| | Error(Time(Trt)) | 4 | 0.22 | 0.54 | | |
| | Class | 6 | 3.15 | 0.53 | 15.84 | 0.0001 |
| | Trt*Class | 6 | 0.08 | 0.01 | 0.40 | 0.873 |
| | Error(Class(Trt)) | 23 | 0.76 | 0.03 | | |
| | Time*Class | 6 | 0.03 | 0.01 | 1.16 | 0.359 |
| | Trt*Time*Class | 6 | 0.06 | 0.01 | 2.11 | 0.091 |
| | Error(Time*Class(Trt)) | 23 | 0.10 | 0.00 | | |

Table 8, continued.

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| **Total Length (mm)** | Trt | 1 | 276.86 | 276.86 | 0.87 | 0.405 |
| | Error(Trt) | 4 | 1,278.57 | 319.64 | | |
| | Time | 1 | 116.10 | 116.10 | 10.01 | 0.034 |
| | Trt*Time | 1 | 4.69 | 4.69 | 0.40 | 0.560 |
| | Error(Time(Trt)) | 4 | 46.38 | 11.59 | | |
| | Class | 6 | 266,566.58 | 44,427.76 | 108.66 | 0.0001 |
| | Trt*Class | 6 | 952.10 | 158.68 | 0.39 | 0.879 |
| | Error(Class(Trt)) | 23 | 9,404.32 | 408.88 | | |
| | Time*Class | 6 | 14.15 | 2.36 | 0.12 | 0.994 |
| | Trt*Time*Class | 6 | 17.25 | 2.87 | 0.14 | 0.989 |
| | Error(Time*Class(Trt)) | 23 | 466.40 | 20.28 | | |

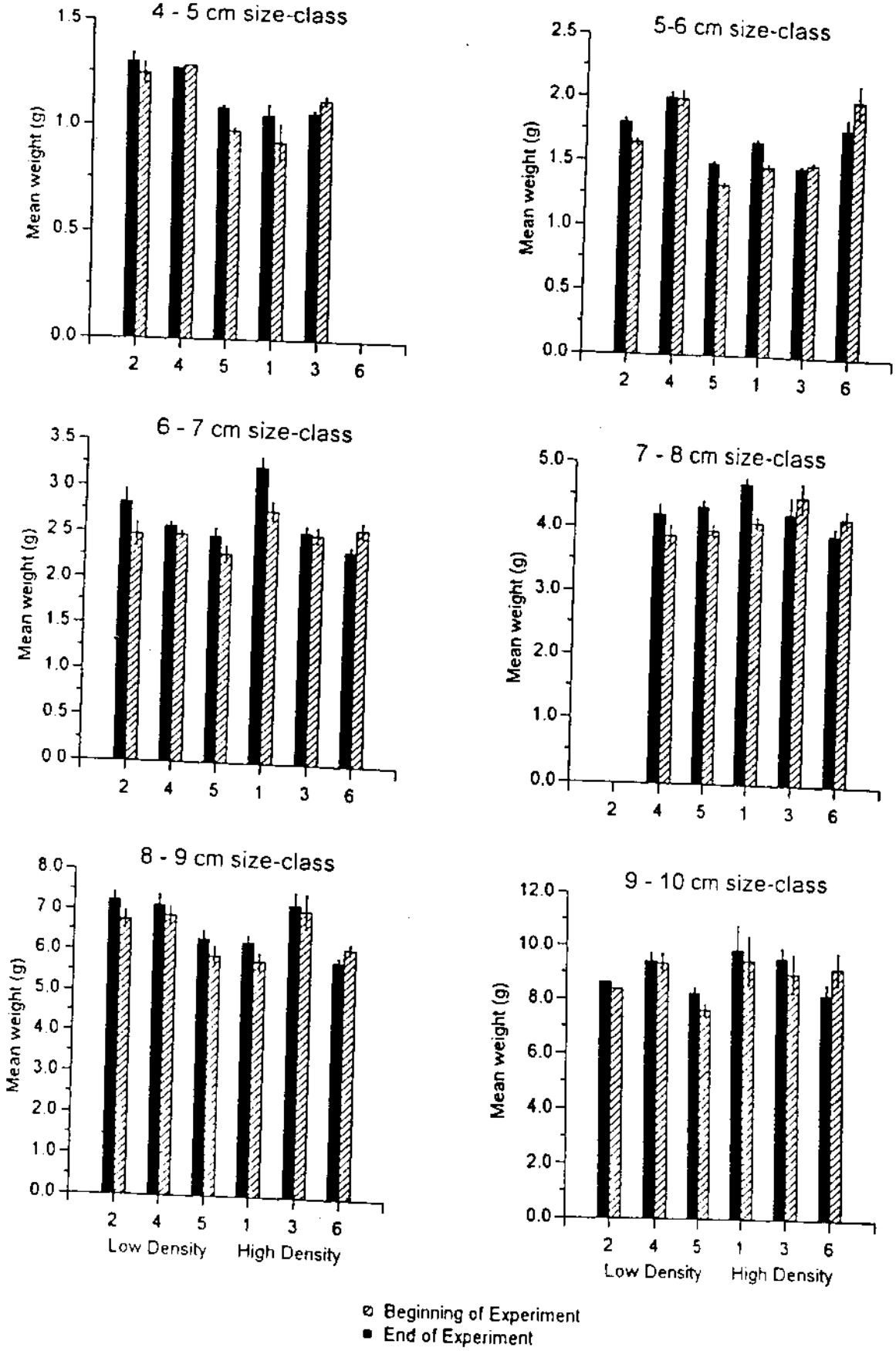


Figure 2a. Mean (± SE) weights (g) of all marked Gila chub, pre- and post- experiment.

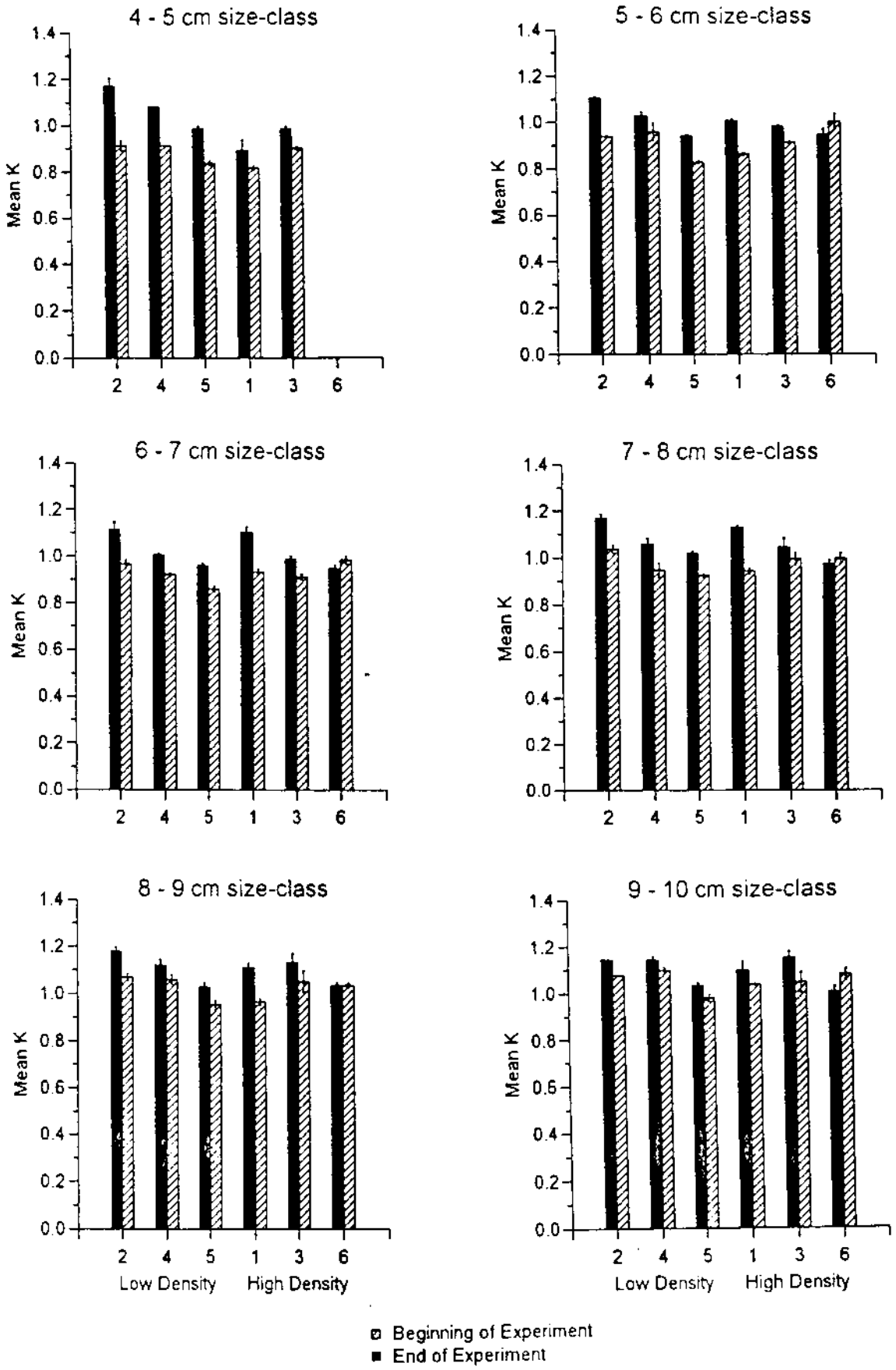


Figure 2b. Mean (± SE) condition factor of all marked Gila chub, pre- and post- experiment.

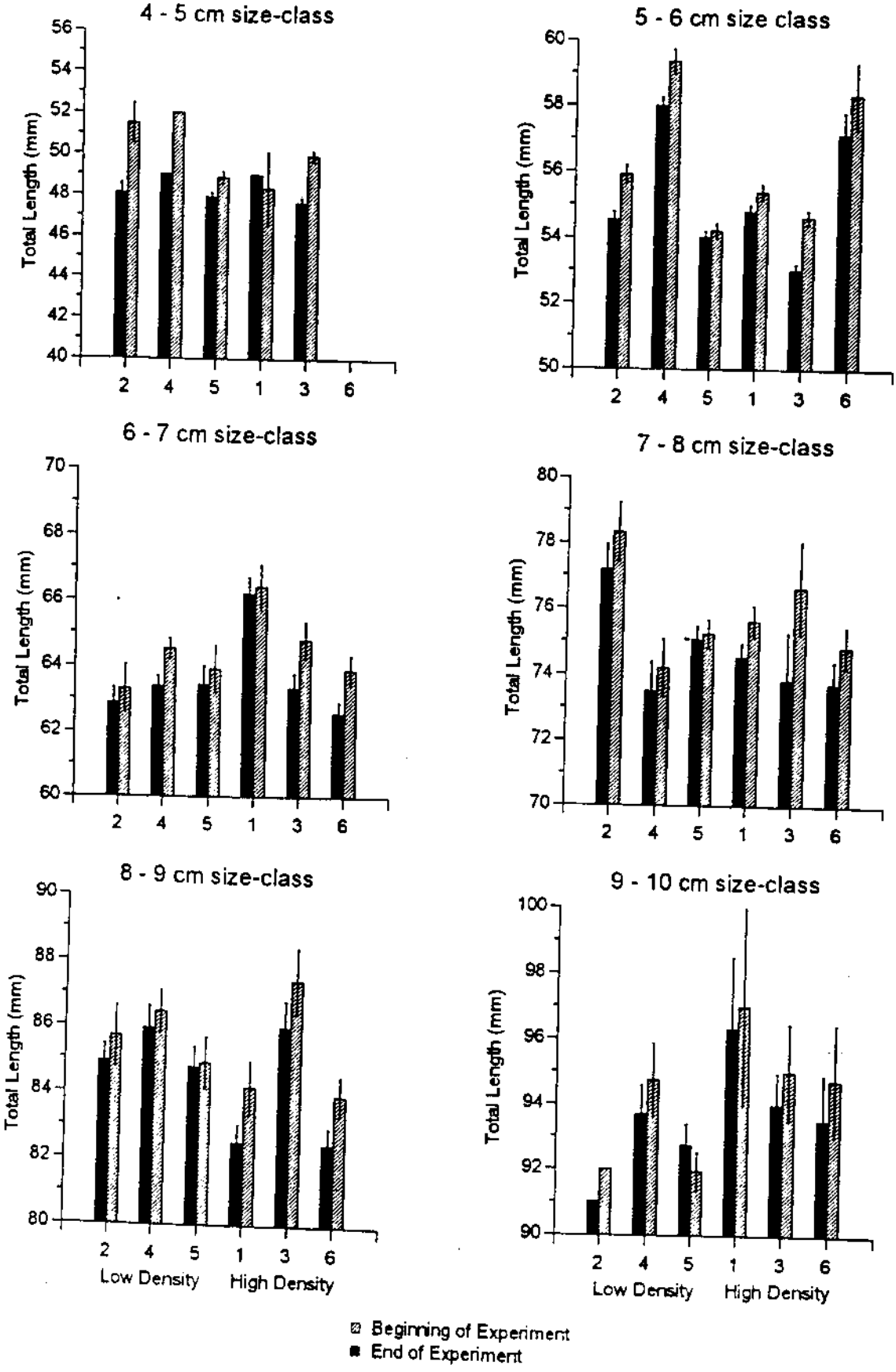


Figure 2c. Mean (± SE) total lengths (mm) of all marked Gila chub, pre- and post- experiment.

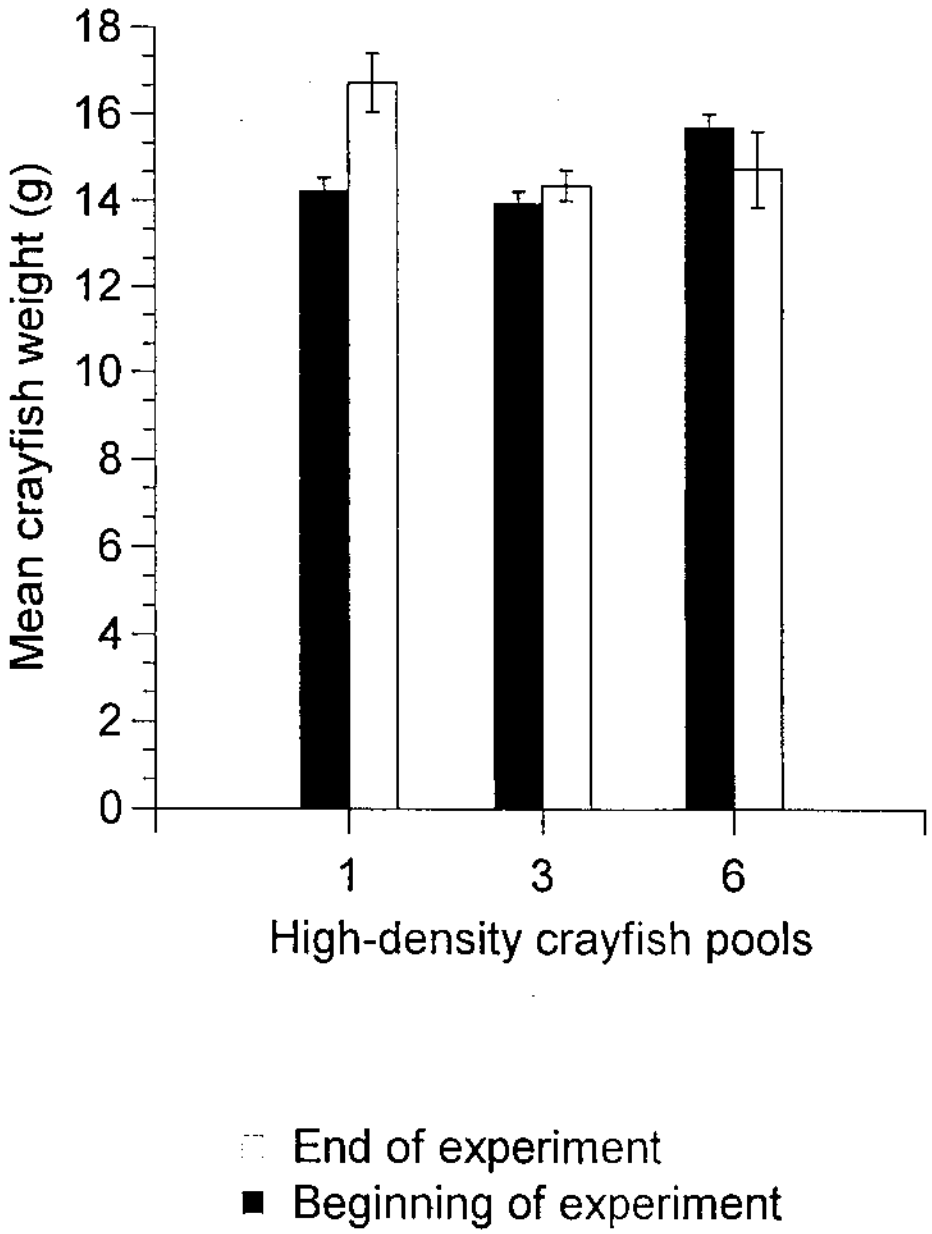


Figure 3. Mean weight of marked crayfish (± SE) in each high-density pool, pre- and post-experiment.

Table 9. Results of analyses of crayfish weight by treatment (low or high density) and time (pre and post-experiment).

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| Mean weight (g) | Pool | 2 | 277.01 | 138.51 | 6.12 | 0.002 |
| | Time | 1 | 62.79 | 62.79 | 2.77 | 0.096 |
| | Time*Pool | 2 | 242.69 | 121.34 | 5.36 | 0.005 |

Table 10. Post-hoc analysis of change in crayfish weight over time for each high-density pool.

| Pool | Mean crayfish weight, g ($\bar{x}$ ± SE, *N*) | | Difference in weight over time, ± SE | *t* | df | *P* |
|---|---|---|---|---|---|---|
| | Pre | Post | | | | |
| 1 | 14.23 ± 0.31, 256 | 16.74 ± 0.68, 59 | -2.52 ± 0.69 | -3.66 | 983 | 0.001 |
| 3 | 13.95 ± 0.28, 291 | 14.39 ± 0.35, 182 | -0.44 ± 0.45 | -0.97 | 983 | 0.330 |
| 6 | 15.71 ± 0.33, 166 | 14.77 ± 0.88, 35 | 0.94 ± 0.89 | 1.06 | 983 | 0.288 |

densities of crayfish in isolated pools. High crayfish densities were expected to facilitate increased interactions between crayfish and fish. However, differences in crayfish densities did not significantly affect average growth rate or condition factor of Gila chub. Based on the growth parameters measured, we saw no evidence of competition between crayfish and Gila chub in shrinking pools in Sabino Creek.

Many authors have reported dramatic effects of exotic species on native fishes of the southwest (e.g., Minckley and Deacon 1968; Moyle 1976; Williams et al. 1989; Minckley and Douglas 1991), and others have reported adverse effects of exotic crayfish upon frogs (Fernandez and Rosen 1996) and invertebrates (Charlebois and Lamberti 1996). In adddition, Dudley (1995) found that Gila chub recruitment is adversely impacted by green sunfish. Therefore, it seems counterintuitive to conclude that crayfish have no effect on growth and condition of Gila chub. It is possible that there are interaction effects between crayfish and Gila chub, but that they are subtle and long-term rather than the acute and overt effects that we attempted to measure in our experiments. It is also possible that crayfish do not impact Gila chub. Crayfish are benthic organisms. In our lab experiments, Gila chub used all levels of the water column equally (see Part III of this report). Gila chub and crayfish may occupy different microhabitats, which may limit their interactions. It may be unrealistic to expect all native fish to respond similarly to introduced species.

Our experiment was of short duration. We measured changes in weight, length, and condition factor over 27-28 days to evaluate competition between crayfish and fish. Many other researchers have successfully observed significant changes in growth in short-duration experiments with similarly-sized animals. Soderback (1994) measured changes in growth in a 35-day experiment to evaluate effects of competition between two crayfish species. Magoulick and Wilzbach (1998) observed changes in growth rates between adults of two trout species in an artificial stream during an 18-day experiment. McMichael et al. (1997) determined hatchery steelhead caused a significant reduction in growth rate of wild juvenile trout (size range: 101-204 mm fork length) over a 42-day experiment. Abrahams (1996) found changes in growth rates of young-of-year minnows in six-week competition experiments. Diehl and Eklov (1995) found differences in growth of juvenile perch (mean initial weights were 2.8-9.0 g) when in the presence of a piscivore in a 44-day field experiment. Persson and Greenberg (1990) saw changes in growth between young *Perca* and *Rutilus* species (averaging 23 and 10 g, respectively) during a two-month field experiment. Mittelbach (1988) conducted a caged experiment and measured growth over 50 days to determine competition between juveniles of two *Lepomis* species (mean caudal length of 50 mm). Cunjak and Green (1986) evaluated competition between juveniles of two trout species by measuring weight change over 10 days.

We marked Gila chub by size-class in this field experiment. Identifying individual fish would have allowed us to compare individual changes in response variable from the beginning to the end of the experiment. We did not want to cause additional stress to Gila chub by subjecting them to more than 2 elastomer injections. These fish are too small for pit-tags, though fingerling tags might have been acceptable (Wydowski and Emery 1983). We concluded that the next best alternative was to mark fish under a narrow size-class interval (1-cm) and evaluate change in response variables for each size class. Indeed, the ANOVA results (overall $P$=0.0001) and Tukey studentized HSD tests found significant differences in all three response variables between all 1-cm size classes except between the two smallest (4-5 and 5-6). Thus the variation within each size-class was less than the variation between size-classes. These ANOVA results provide evidence that our size-class intervals were appropriate for describing changes in response variables.

Our experiment used pools in a drying stream as units of replication. Using pools as replicate units is an accepted approach in experimental design for both observational and experimental studies. Deegan et al. (1997) used riffle/pool combinations as treatment units to assess effects of fish density and river fertilization on algal standing stocks, invertebrate communities, and fish production in an arctic river. Fraser and Gilliam (1992) used replicate pools in a Trinidad stream to determine the effect of a fish predator on habitat use by 2 prey fish species. Gelwick and Matthews (1992) used 8 stream pools as replicates in a fish grazing experiment in Oklahoma. Harvey and Stewart (1991) used individual pools as units of replication in a predation experiment in 3 small streams in eastern Tennessee. Capone and Kushlan (1991) made inferences about factors influencing community structure from observations in 40 dry-season pools in a hydrologically variable river drainage in northeast Texas.

We focused on conducting a thorough sample of six replicate pools in this experiment. The apparent absence of treatment effects may be due to lowered statistical power, either because of the small number of replicate pools (Eberhardt and Thomas 1991) or because of the significant interaction effect between pools and treatments. A longer experimental period may have also given us better resolution of interaction effects. However, earlier work with Sonora sucker (*Catostomus insignis*) indicated that during a similar pre-monsoon period in 1995, fish lost up to 10% of their body mass in only two weeks' time (J. Carpenter, unpublished data). In addition, the high consistency among response variables between pools, especially for mean weight and condition factor (Figures 2a and 2b), suggests that low statistical power may not be an obstacle towards observing a significant effect in this experiment. It is also possible that crayfish-Gila chub interactions are more subtle and complex than could be identified in our simple experiments. We suggest that competition effects should be explored in a laboratory where there is more

control of extraneous variables (changes in water level, differences in water quality and habitat, or movement of crayfish and fish between pools).

Due to logistical and personnel constraints, we were unable to weigh, mark, and measure all Gila chub and crayfish in one day; therefore we were unable to begin and end the experiment at all six pools simultaneously. Instead we had paired trials of 4-week durations over a 12-week period. Undoubtedly stream conditions in the earlier trials differed from those in the later trials. It is possible that varying conditions between time periods differentially affected fish growth. Low levels of dissolved oxygen was the only water chemistry variable that was significantly different among treatments, and we found no differences in habitat variables between treatments.

The elastomer used for marking fish in this experiment was recently used by Haines and Modde (1996) on age-0 Colorado squawfish (*Ptychocheilus lucius*). They found low mortality rates (5-12%) and no differences in predation vulnerability or growth between marked and unmarked fish. Assuming that Gila chub respond to marking as did Colorado squawfish, there is no evidence that the decrease in biomass we observed among marked Gila chub was due to marking technique. Rather, the observed weight loss in free-ranging fish is more likely indicative of physiological stress (Bonga 1997). We know of no data on the periodicity of growth of this or analogous small native fishes in Sonoran desert streams. We hypothesize that Gila chub may respond to decreasing stream flow and associated isolation and shrinkage of pools by lowering their metabolic rate such that little or no growth occurs during these physiologically stressful times.

We had originally proposed to collect relative abundance data on macroinvertebrates within each pool. We attempted various sampling methods such as emergence traps (see Merritt and Cummins 1996 for description), kick sampling, and the benthic sampler used at the Three Forks site (see Part II of this report); however, these techniques did not work at Sabino. The emergence traps and kick samples produced extremely low numbers of invertebrates; also the latter technique is difficult to replicate and quantify. The benthic sampler was ineffective due to low flows and inappropriate substrate types (primary substrates in Sabino Creek are sand, boulder, and bedrock). Dr. Stuart Fisher (pers. comm., April 1996, Arizona State University) recommended using artificial substrates in these types of habitats, but they would have been easy targets for vandals and curious visitors to this popular recreation area. Therefore we were forced to omit the invertebrate work from the Sabino field study.

Nearly all size classes of Gila chub were well-represented within our experimental pools, regardless of crayfish density. All pools but Pool 6 contained some 40-49 mm fish and Pools 1, 2, 3, and 5 contained high numbers of the 50-59-mm size-class pre-and post-experiment, which suggests successful

recruitment. If we assume that our recapture rates (Table 7) reflect survivorship, the high recapture rates indicate high densities of crayfish had no effect on Gila chub mortality. Crayfish do not appear to be affecting Gila chub population structure in Sabino Canyon, at least not on the same scale as green sunfish affect Gila chub (Dudley 1995).

Food resources available to Gila chub may not have had time to recover in the four weeks after crayfish densities were established. It is also possible that intraspecific competition among Gila chub had a greater effect than interspecific competition (Begon and Mortimer 1987) with crayfish.

Our fish growth data must be interpreted carefully. Fish were not individually tagged; therefore it is not possible to determine a weight change for an individual fish. For instance, if the largest fish in a given size class were captured at the beginning of the treatment, grew significantly during the course of the experiment, but escaped capture at the end, then we might falsely assume that there was a decrease in mean weight over time for that size class. On the other hand, if the smallest fish in a given size-class died over the course of the experiment, then mean weight for that size-class would have increased and we might falsely assume a positive effect. However, high recapture rates do not support either scenario.

Pool 6 was dissimilar in abiotic (chemistry, morphology) and biotic parameters (fish and crayfish densities) from all other pools. In Pool 6 crayfish numbers decreased 65% over the course of the experiment. This pool was at the top of a large, dry water fall. Near the end of the experiment we found carcasses of several crayfish from Pool 6 at the bottom of the falls; apparently the crayfish chose to traverse over the dry fall rather than remain in Pool 6. It appears that large numbers of crayfish left the pool over the course of the experiment. The general shape of graphs in Figure 2c indicates that the fish population in Pool 6 also differed from the others in terms of total lengths of each cohort. However, the change between pre- and post- experiment weights and lengths of fish in Pool 6 do not appear different from the other pools. Removing Pool 6 and re-analyzing the fish response variables produced no difference in the results or significance of the ANOVA tests. It is doubtful that the data from Pool 6 altered the results of this study.

In summary, average change in Gila chub weight, condition factor, and total length did not differ between high-density and low-density crayfish sites. In addition, crayfish weights did not change over time with respect to pools or treatment. Therefore, our experiment found no evidence of competition for forage between crayfish and Gila chub. We can not reject our null hypothesis based on the results of our statistical analyses. However, not rejecting the null hypothesis does not mean that we can conclude crayfish do not compete with Gila chub. Rather, we suggest that further research needs to be conducted

to evaluate the interactions between Gila chub and crayfish, preferably under a setting where extraneous variables can be more easily controlled than under the field conditions we experienced.

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# EFFECTS OF AN INTRODUCED CRAYFISH (*ORCONECTES VIRILIS*) ON NATIVE FISHES AND MACROINVERTEBRATES AT THREE FORKS, ARIZONA

Jeanette Carpenter

and

Carole McIvor

Arizona Cooperative Fish and Wildlife Research Unit
Bio Sciences East #104, University of Arizona, Tucson, AZ 85721

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**Effects of an Introduced Crayfish (*Orconectes virilis*) on Native Fishes and Macroinvertebrates at Three Forks, Arizona**

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## ABSTRACT

We employed a field experiment during fall 1996 to examine effects of an exotic crayfish, *Orconectes virilis*, on benthic macroinvertebrates, *Ranunculus aquatilis* and associated invertebrates, and three native fishes in a small unnamed stream at Three Forks in the White Mountains, Arizona. Because *O. virilis* is polytrophic, it can potentially compete for forage with all three fish species: speckled dace (*Rhinichthys osculus*), Sonora sucker (*Catostomus insignis*), and desert sucker (*C. clarkii*). Eight stream sections were fenced with wiers to prevent crayfish and fish movement. We randomly assigned the eight sites to two treatments: low-density and high-density crayfish sites. In low-density sites, we removed as many crayfish as possible. In high-density sites, we re-introduced crayfish at a mean density of 1.7 individuals/$m^2$. *Ranunculus aquatilis* biomass was significantly reduced in high-density sites compared to low-density sites. Molluscs $>10$ mm were entirely absent from *R. aquatilis* samples in high-density sites. There were no other differences among other invertebrates associated with the aquatic plant. Benthic samples had lower insect diversity in high-density sites compared to low-density sites, but we saw no significant differences in invertebrate abundance between treatments. There was no treatment effect as measured by the relative change in biomass and condition factor among individually marked fish. Speckled dace showed an increase in biomass and condition factor at all sites, whereas suckers declined in weight and condition in at least one site. Based on fish response, we could not reject the null hypothesis that crayfish do not compete with these native fish for forage at Three Forks.

## INTRODUCTION

Crayfish occur throughout the contiguous United States. However, in Arizona they are not native (Hobbs 1988). At least two species now occur in Arizona waters: *Orconectes virilis* and *Procambarus clarkii* (Taylor et al. 1996; Inman et al. 1998). It is unclear how or when crayfish first invaded Arizona streams; they were probably introduced by agencies or individuals for the purpose of

providing forage or bait for game fish. One of the first scientific reports to mention the presence of crayfish in Arizona (Dean 1969) reports their occurrence in Nutrioso Creek in the White Mountains[1].

Crayfish can greatly impact the ecology of streams to which they are not native. Fernandez and Rosen (1996) studied crayfish in an unnamed tributary of the East Fork of the Black River at Three Forks (White Mountains) and determined (from stream sampling and lab experiments) that crayfish negatively impacted aquatic vegetation, macroinvertebrates, and the Chiricahua leopard frog (*Rana chiricahuensis*). Native fishes occurring at Three Forks may also be threatened by increased numbers of crayfish. Therefore, we decided to conduct a field experiment to determine effects of *Orconectes virilis* on three native fishes and the invertebrate forage base at Three Forks.

The native fish species found at Three Forks are speckled dace (*Rhinichthys osculus*), desert sucker (*Catostomus clarkii*), and Sonora sucker (*C. insignis*). The Sonora sucker is a generalized carnivore and feeds primarily on aquatic invertebrates whereas the desert sucker is highly algivorous (Schreiber and Minckley 1981), but can be seasonally detritivous or insectivorous (Gregor and Deacon 1988). Speckled dace primarily consume aquatic insects, specifically small dipteran and ephemeropteran larvae (Gregor and Deacon 1988). *Orconectes virilis* is omnivorous and can feed at several trophic levels (Momot et al. 1978; Hobbs 1993). Therefore, it may compete for food with all three fish species.

The objectives of this study were to determine if presence of high numbers of crayfish reduced growth rate and condition factor of speckled dace, Sonora sucker, and desert sucker, diversity and abundance of macroinvertebrates, and biomass of submerged aquatic vegetation.

## Study area

The field experiment was conducted within an unnamed perennial tributary of the East Fork of the Black River at Three Forks in the Apache Sitgreaves National Forest. Three Forks is approximately 19 km west of the town of Alpine (SE Section 6 and SW Section 5, T5N R29E; Figure 1). The unnamed stream (henceforth called Three Forks) follows FR 249 to the south at an elevation of 2506 m (8220 feet) and meanders through a grassy meadow throughout the study reach.

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[1] Although Dean (1969) refers to the crayfish species in Nutrioso Creek as *O. causeyi*, Hobbs believed it was *O. virilis* (Momot et al. 1978). Hobbs considered the two species to be indistinguishable and referred to *O. causeyi* as a synonym for *O. virilis* (Hobbs 1972, 1989). Inman et al. (1998) used the measurement techniques of Unger (1978) to determine that the crayfish in Nutrioso Creek is *O. virilis*

It is a very small first-order stream with an average width of 2.4 m and an average depth of <0.3 m (Table 1).

## METHODS AND MATERIALS

We conducted a field experiment to determine effects of crayfish on native fish, aquatic invertebrates, and submerged aquatic vegetation. We identified eight stream sections and manipulated crayfish numbers in each section, so that four had low densities of crayfish and four had high densities of crayfish. The eight stream sections were treated as replicate sampling units. Each section included both pools and riffles. We first estimated surface area of chosen sites and conducted a preliminary electrofishing sample to confirm the use of this habitat by the target fish and crayfish. To restrict movement between sections, we built weirs out of inert aquaculture netting that had 1/4-inch (6 mm) openings. The netting was 1.2 m high and held to the substrate with rebar and sandbags filled with gravel. The wiers were extended laterally into the streambank to prevent washouts (Deegan et al. 1997). Weirs were maintained by consistent (every 2-4 days) surveillance, repair and cleaning. We found low numbers of non-native trout (*Salmo trutta, Salvelinus fontinalis*) in this stream. These individuals were removed from our experimental sections and transferred downstream before our experiments began.

### Habitat measurements

Instream habitat data were collected on 4-9 October. Habitat data were obtained from the eight stream sections by establishing transects two meters apart perpendicular to stream flow, and measuring depth and velocity every 0.2 meters along these transects. We also visually estimated the dominant substrate type (silt, sand, gravel, cobble, boulder, roots, vegetation) at each measurement location. We measured current velocity with a Pigmy flow meter attached to a steel wading rod, which we also used to determine water depth to an accuracy of 0.1 ft (3 cm). Dissolved oxygen, conductivity, and temperature were measured with a YSI meter; pH was determined with a Corning Checkmate water chemistry instrument.

Table 1. Physical habitat of experimental sites at Three Forks, sites measured on 4 - 9 October 1996.

| Site | Treatment | Surface Area ($m^2$) | Mean Depth (cm) | Mean Velocity (cm/sec) | Relative abundance of substrates (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Silt <0.06 mm | Sand 0.06-2 mm | Gravel 2-64 mm | Cobble 64-256 mm | Boulder >256 mm | Vegetation |
| 1 | High-density | 47.2 | 26.0 | 0.81 | 26 | 0 | 53 | 0 | 0 | 21 |
| 2 | High-density | 32.4 | 23.1 | 1.92 | 56 | 1 | 21 | 2 | 0 | 21 |
| 3 | Low-density | 20.0 | 9.4 | 5.35 | 31 | 0 | 50 | 0 | 0 | 19 |
| 4 | Low-density | 34.1 | 21.0 | 2.60 | 33 | 0 | 42 | 0 | 1 | 24 |
| 5 | High-density | 50.2 | 24.9 | 2.23 | 34 | 0 | 35 | 0 | 0 | 31 |
| 6 | Low-density | 23.8 | 16.5 | 4.37 | 29 | 0 | 31 | 0 | 0 | 40 |
| 7 | Low-density | 43.0 | 14.5 | 1.17 | 14 | 1 | 52 | 0 | 1 | 32 |
| 8 | High-density | 32.6 | 19.5 | 1.62 | 23 | 0 | 54 | 0 | 2 | 21 |

Benthic invertebrates

Benthic invertebrate samples were collected with a modified Hess sampler (Hess 1941) at the beginning of the field experiment, from 18-25 September and at the end from 2-6 November. The sampler had a diameter of 358 mm, thus it sampled 1,007 $cm^2$ of substrate. In each of the 8 stream sections, we collected 4 invertebrate samples. Sample locations within a given section were determined with a stratified random design: two random samples within pool habitat, and two random samples within riffle/run habitat. After pushing the sampler into the substrate, we noted most abundant substrate type, presence of aquatic vegetation, and percent embeddedness of substrate in quartiles, as described by Bovee (1986). The substrate was disturbed for 60 seconds, in which time we rubbed all stones within the circumference of the delimited sampling area, and flushed floating invertebrates into the collection bag of the sampler (mesh size of 420 $\mu$m). When the sampler was still in place we measured water depth at four equidistant points around the sampler (following ordinal direction; i.e., N, S, E, and W points). Benthic samples were poured over a brass #40 sieve (mesh size approximately 450 $\mu$m) to remove sediment before transferring into jars of 90% ethanol. In the laboratory we picked samples carefully and sorted and counted macroinvertebrates: by order for aquatic insects and by class for all other organisms.

*Ranunculus aquatilis* samples

Fernandez and Rosen (1996) observed *Orconectes virilis* grazing heavily upon *Ranunculus aquatilis* in this stream. In lab experiments they showed that biomass of *R. aquatilis* decreased 81% in aquaria containing crayfish. We chose to approach this interaction from a different perspective by testing crayfish effects within the stream. On 2 October we collected samples of *R. aquatilis* from an area downstream of our study site. We filled equally 27 aluminum pie pans (dimensions were 20-cm diameter X 4-cm high) with the samples, carefully keeping roots attached to the substrate when filling the pans. We randomly assigned three pans to each stream section, making up a total of 24 pans in the stream. The three additional samples were collected to estimate the amount of vegetation and invertebrates at the beginning of the experiment. During the experiment *R. aquatilis* samples were checked to make sure that the vegetation remained alive, and to determine if there was any obvious use by crayfish or fish. On 5 November, samples were removed from the stream and transferred into zip-lock bags filled with 90% ethanol. In the lab each sample was carefully washed over a brass #40 sieve to remove sediment and attached invertebrates. Invertebrates were collected and placed in jars

of alcohol. Plant material was drained on a #40 brass sieve, placed in paper bags, oven dried for 24 hours at 70°C, and weighed to determine oven-dried biomass.

Fish and crayfish

We used unbaited minnow traps and electrofishing to capture fish. We measured total length and weight of all captured fish, and marked individuals > 4 cm with fluorescent elastomere (Haines and Modde 1996) in a way that allowed us to identify individual fish. Fish were captured and measured from 2-24 September, and recaptured and weighed at the end of the experiment from 9-13 November. The start dates of stream sites were staggered slightly due to the time necessary to set up each site. Fish remained in the experiment from 44 to 71 days. In analyzing the data, we accounted for the different times fish were in the experimental conditions by transforming the raw biomass and length data to weight change per day, then multiplying by 44, which was the minimum length of time a fish was in the experiment. In using this standardization method we assume that the change in response variables was linear. Our data from a similar study on Sonora sucker indicates a direct relationship between weight change in the first two weeks of an experiment and the change in the second two weeks of the experiment ($r^2$=0.69, $N$=12, $P$=0.001; Carpenter and McIvor 1998).

We primarily used baited minnow traps to capture crayfish, but we also collected any crayfish encountered during electrofishing. Crayfish were captured from 22 August through 18 September and kept in large cages until we obtained depletion estimates at each site, after which we measured and marked them prior to release on 19-25 September. We measured carapace lengths (CL) and weighed crayfish, and marked them with pleural clips (Goellner 1943) to indicate the stream section into which we released them. Crayfish were recaptured and measured again at the end of the experiment, from 9-13 November. During the course of the experiment we continued to trap to ensure that crayfish densities remained high in the high-density sites, and that we depressed if not depleted crayfish in the low-density sites. This study was conducted in autumn; therefore we noted activity levels of crayfish and fish as water temperatures decreased.

Statistical analysis

This experiment was a randomized factorial design. To compare fish growth among treatments, we used a multiple-factor ANOVA in which effects were treatment (low- or high-density crayfish), sites, and time (pre- and post-experiment). Fish weight and Fulton's condition factor (Bagenal and Tesch 1978), or K (100,000 X g/mm$^3$) were the fish response variables. Other

dependent variables were characteristics of benthic invertebrates from Hess and *R. aquatilis* samples (diversity, total abundance, and abundance of selected taxa), and biomass of *R. aquatilis*. Each response variable was first tested for site effects, which were nested within treatments. A significant result for sites nested within crayfish treatment indicated that there was a site effect; if this occurred we collapsed the data and compared the response variable at the level of mean response for each site. Thus sites rather than individual observations within sites would become the units of replication. We used an $\alpha$-level of 0.05 to determine significance of statistical tests.

## RESULTS

### Habitat measurements

Our replicate stream sites were fairly similar in terms of surface area and velocity (Table 1; paired comparison t-tests: $P > 0.1$) but varied in depth. Low-density sites were slightly shallower ($\bar{x} = 15.34$ cm $\pm$ 2.4 SE) than high-density sites ($\bar{x} = 23.35$ cm $\pm$ 1.42 SE; paired comparison t-test: $t = -2.87$, df=6; $P = 0.036$). Water chemistry was similar among treatments (Table 2; paired comparison t-tests: $P > 0.1$). Water temperatures within Three Forks dropped over time as autumn progressed (Figure 2). Crayfish and fish appeared less active during brief periods when temperatures were low.

### Benthic invertebrates

Actual abundances of invertebrates by taxon are provided in Appendix A. Total abundance of macroinvertebrates (excluding crayfish, Acarina, amphipods, and ostracods) was much higher in riffles than in pools (Figure 3). Therefore we analyzed the pre- and post-experiment data separately by habitat type. Sample sizes were 8 samples/time/treatment from pools and 7-8 samples/time/treatment from riffles. To determine if crayfish treatment affected invertebrate numbers, the main factor we are interested in is the Trt*Time interaction (Tables 3 and 4): this factor determines if our response variables differed between treatments over time. We compared five different characteristics of the benthic community: total abundance, diversity of insects, abundance of Trichopterans and molluscs, and numbers of molluscs > 10 mm.

Table 2. Water chemistry of experimental sites at Three Forks. Measurements on any given day were taken at all sites within a two-hour time span.

| Site | Treatment | Water Temperature (°C) | DO mg/L | | TDS mg/L | | pH | |
|---|---|---|---|---|---|---|---|---|
| | | 3 Oct. | 26 Sept. | 12 Nov. | 26 Sept | 3 Oct. | 26 Sept | 3 Oct. |
| 1 | High-density | 14.2 | 9.2 | 10.1 | 61.1 | | | 7.7 |
| 2 | High-density | 14.5 | 10.2 | | 55.9 | | | 8.1 |
| 3 | Low-density | 14.7 | 9.2 | | 55.0 | | | 8.1 |
| 4 | Low-density | 15.2 | 9.1 | | 53.8 | | | 7.8 |
| 5 | High-density | 15.7 | | | | 57.9 | | 8.0 |
| 6 | Low-density | 15.7 | | | | 59.1 | | 7.8 |
| 7 | Low-density | 15.9 | | 10.1 | | 59.1 | | 8.0 |
| 8 | High-density | 16.1 | | | | 58.5 | | 7.8 |

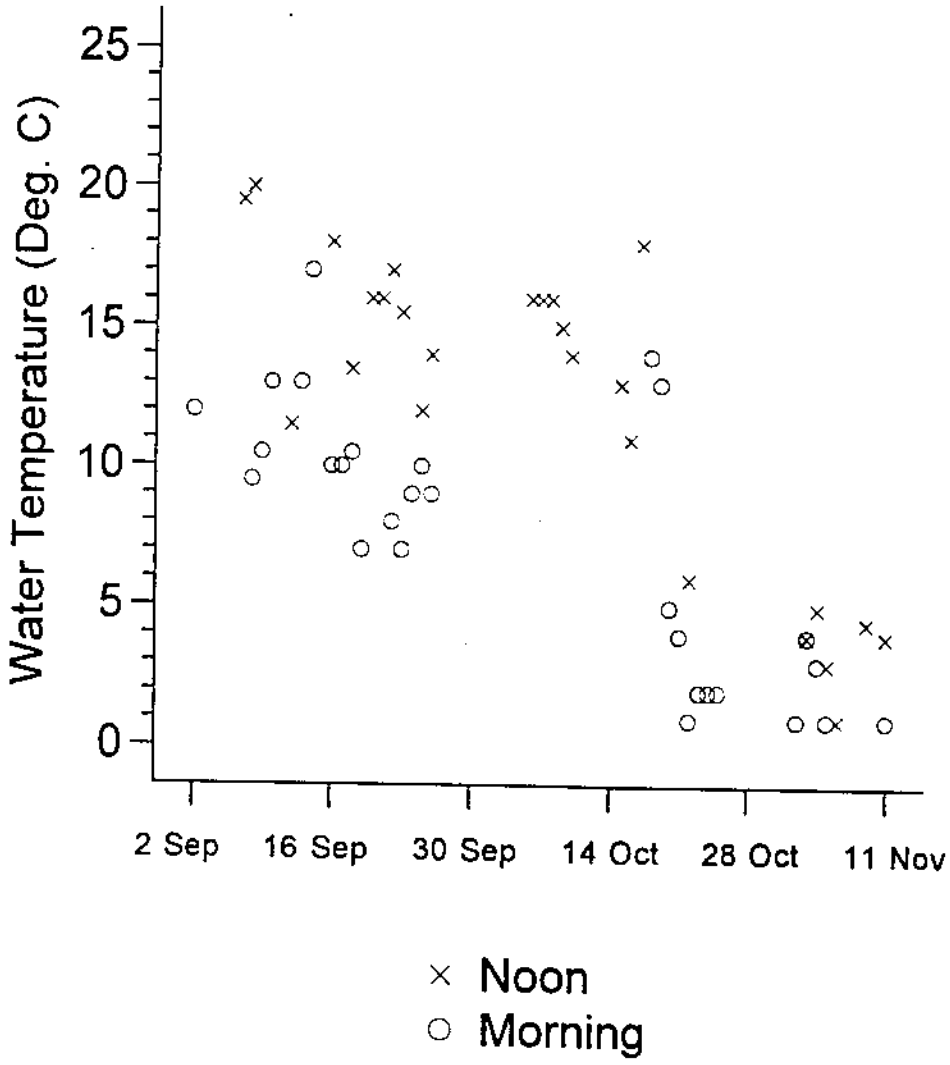


Figure 2. Water temperatures measured at Three Forks in the morning (8:00-10:00 hours) or at noon (12:00-13:00 hours).

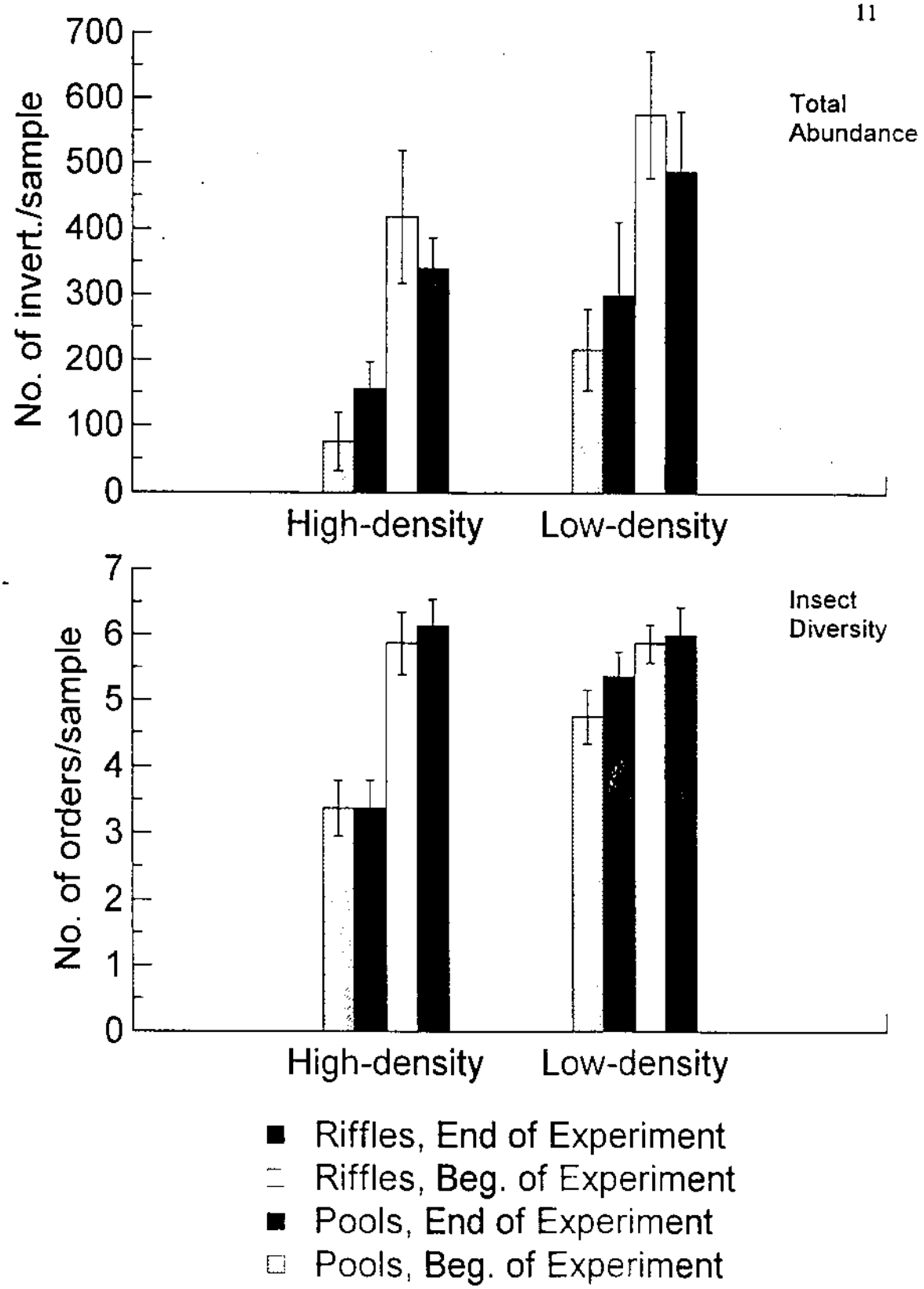


Figure 3. Mean (± SE) abundance of invertebrates and insect diversity (number of orders/sample) in Hess samples collected from pools and riffles in sites of high-density and low-density crayfish treatments.

Table 3. Summary of analyses of variance testing effects of crayfish treatment (low or high density), time (pre and post-experiment) and their interaction on benthic macroinvertebrates collected in pools. Site was used as a nesting factor.

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| POOLS: Total invertebrate abundance | Trt | 1 | 162307.53 | 162307.53 | 1.36 | 0.287 |
| | Error(Trt) | 6 | 715424.44 | 119237.41 | | |
| | Time | 1 | 53546.28 | 53546.28 | 2.31 | 0.179 |
| | Trt*Time | 1 | 26.28 | 26.28 | 0.01 | 0.974 |
| | Error(Time(Trt)) | 6 | 139026.19 | 23171.03 | | |
| Insect diversity (number of orders/sample) | Trt | 1 | 22.78 | 22.78 | 11.95 | 0.014 |
| | Error(Trt) | 6 | 11.44 | 1.91 | | |
| | Time | 1 | 0.78 | 0.78 | 0.57 | 0.478 |
| | Trt*Time | 1 | 0.78 | 0.78 | 0.57 | 0.478 |
| | Error(Time(Trt)) | 6 | 8.19 | 1.36 | | |
| Trichopteran abundance | Trt | 1 | 60.50 | 60.50 | 6.74 | 0.041 |
| | Error(Trt) | 6 | 53.88 | 8.98 | | |
| | Time | 1 | 8.00 | 8.00 | 0.88 | 0.384 |
| | Trt*Time | 1 | 3.13 | 3.13 | 0.34 | 0.579 |
| | Error(Time(Trt)) | 6 | 54.38 | 9.06 | | |
| Mollusc abundace | Trt | 1 | 13.78 | 13.78 | 2.24 | 0.185 |
| | Error(Trt) | 6 | 36.94 | 6.16 | | |
| | Time | 1 | 7.03 | 7.03 | 1.08 | 0.338 |
| | Trt*Time | 1 | 5.28 | 5.28 | 0.81 | 0.402 |
| | Error(Time(Trt)) | 6 | 38.93 | 6.49 | | |
| Abundance of Molluscs > 10 mm | Trt | 1 | 0.28 | 0.28 | 2.45 | 0.168 |
| | Error(Trt) | 6 | 0.69 | 0.11 | | |
| | Time | 1 | 0.28 | 0.28 | 2.45 | 0.168 |
| | Trt*Time | 1 | 0.28 | 0.28 | 2.45 | 0.168 |
| | Error(Time(Trt)) | 6 | 0.69 | 0.11 | | |

Table 4. Summary of analyses of variance testing effects of crayfish treatment (low or high density), time (pre and post-experiment) and their interaction on benthic macroinvertebrates collected in riffles. Site was used as a nesting factor.

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| **RIFFLES:** | Trt | 1 | 156156.69 | 156156.69 | 1.75 | 0.234 |
| **Total invertebrate abundance** | Error(Trt) | 6 | 534747.45 | 89124.58 | | |
| | Time | 1 | 81320.03 | 81320.03 | 1.54 | 0.261 |
| | Trt*Time | 1 | 536.69 | 536.69 | 0.01 | 0.923 |
| | Error(Time(Trt)) | 6 | 317182.82 | 52863.80 | | |
| **Insect diversity (number of orders/sample)** | Trt | 1 | 0.11 | 0.11 | 0.05 | 0.834 |
| | Error(Trt) | 6 | 13.95 | 2.33 | | |
| | Time | 1 | 0.11 | 0.11 | 0.17 | 0.692 |
| | Trt*Time | 1 | 0.11 | 0.11 | 0.17 | 0.692 |
| | Error(Time(Trt)) | 6 | 3.86 | 0.64 | | |
| **Trichopteran abundance** | Trt | 1 | 342.25 | 342.25 | 0.60 | 0.467 |
| | Error(Trt) | 6 | 3401.05 | 566.84 | | |
| | Time | 1 | 132.25 | 132.25 | 0.32 | 0.591 |
| | Trt*Time | 1 | 0.03 | 0.03 | 0.00 | 0.994 |
| | Error(Time(Trt)) | 6 | 2465.59 | 410.93 | | |
| **Mollusc abundace** | Trt | 1 | 8.03 | 8.03 | 0.20 | 0.671 |
| | Error(Trt) | 6 | 241.41 | 40.23 | | |
| | Time | 1 | 2.25 | 2.25 | 0.21 | 0.664 |
| | Trt*Time | 1 | 1.36 | 1.36 | 0.13 | 0.735 |
| | Error(Time(Trt)) | 6 | 64.77 | 10.80 | | |
| **Abundance of Molluscs > 10 mm** | Trt | 1 | 0.69 | 0.69 | 6.55 | 0.043 |
| | Error(Trt) | 6 | 0.64 | 0.11 | | |
| | Time | 1 | 0.25 | 0.25 | 0.97 | 0.363 |
| | Trt*Time | 1 | 0.25 | 0.25 | 0.97 | 0.363 |
| | Error(Time(Trt)) | 6 | 1.55 | 0.26 | | |

In pool habitats, insect diversity was higher in low-density pools than in high density pools before the experiment, and this difference remained throughout the experiment (Figure 4; Table 3; $P$=0.014). Similarly, numbers of trichopterans were higher in low-density vs. high-density pools, regardless of time (Figure 4, Table 3: $P$=0.041). In other words, numbers of trichopterans differed between treatments before the experiment began. In riffle habitats, there was no difference in treatment and time for total abundance of invertebrates, insect diversity, or in trichopteran and mollusc abundance (Figures 3-5, Table 4).

In analyzing the invertebrate data from Hess samples, we saw no significant interaction between treatment and time. Therefore, we saw no evidence that high crayfish densities altered benthic invertebrate abundance or diversity. However, larger snails and mussels (>10 mm) were not found in Hess samples from high-density areas at any time (Figure 5), and only 1 large mollusc was found in all 8 low-density samples collected before the treatments began. At the end of the experiment, large molluscs were found in low numbers in riffles and pools in low-density sites; this difference between treatments was significant for riffle samples (Table 4: $P$=0.043).

*Ranunculus aquatilis* samples

Samples of *R. aquatilis* from low-density crayfish sites had higher biomass than samples from high-density crayfish sites, although the difference had marginal statistical significance (Figure 6; Table 5: $P$=0.074). Oven-dried samples from low-density sites averaged 5.5 g more than from high-density sites (Figure 6).

Total number and relative abundances of the most common invertebrates in *R. aquatilis* did not differ between treatments (Figure 7; Table 6). Actual abundances of invertebrates by taxon are provided in Appendix B. There was no difference in number of trichopteran larvae or in total number of molluscs between treatments. However, we did find a significant difference in numbers of larger molluscs. Snails and mussels larger than 10 mm were entirely absent from high-density sites, but averaged 1.9 (± 0.61 SE) individuals per sample in low-density sites (Figure 8; paired comparison t-test: $t$=2.75, df=10, $P$=0.020).

Crayfish density and biomass

Before the experiment began, we found an average of 1.83 crayfish/m$^2$ among all sites. On 19 September we stocked sites with crayfish in the densities shown in Table 7. Densities in high-density sites varied from 1.36-2.16 crayfish/m$^2$ at the beginning of the experiment. Originally, we had

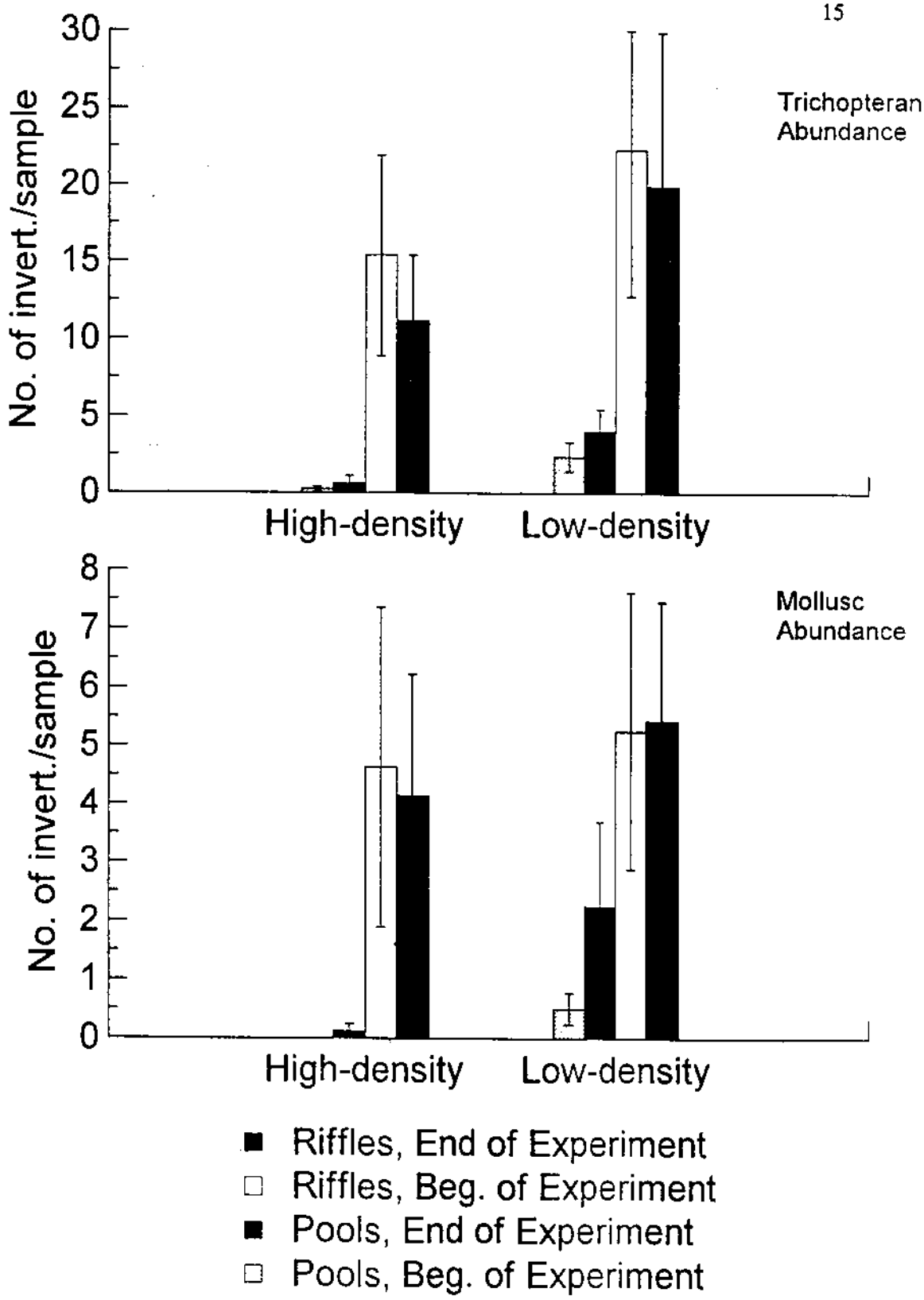


Figure 4. Mean (± SE) abundance of trichopterans and molluscs in Hess samples collected from pools and riffles in sites of high-density and low-density crayfish treatments.

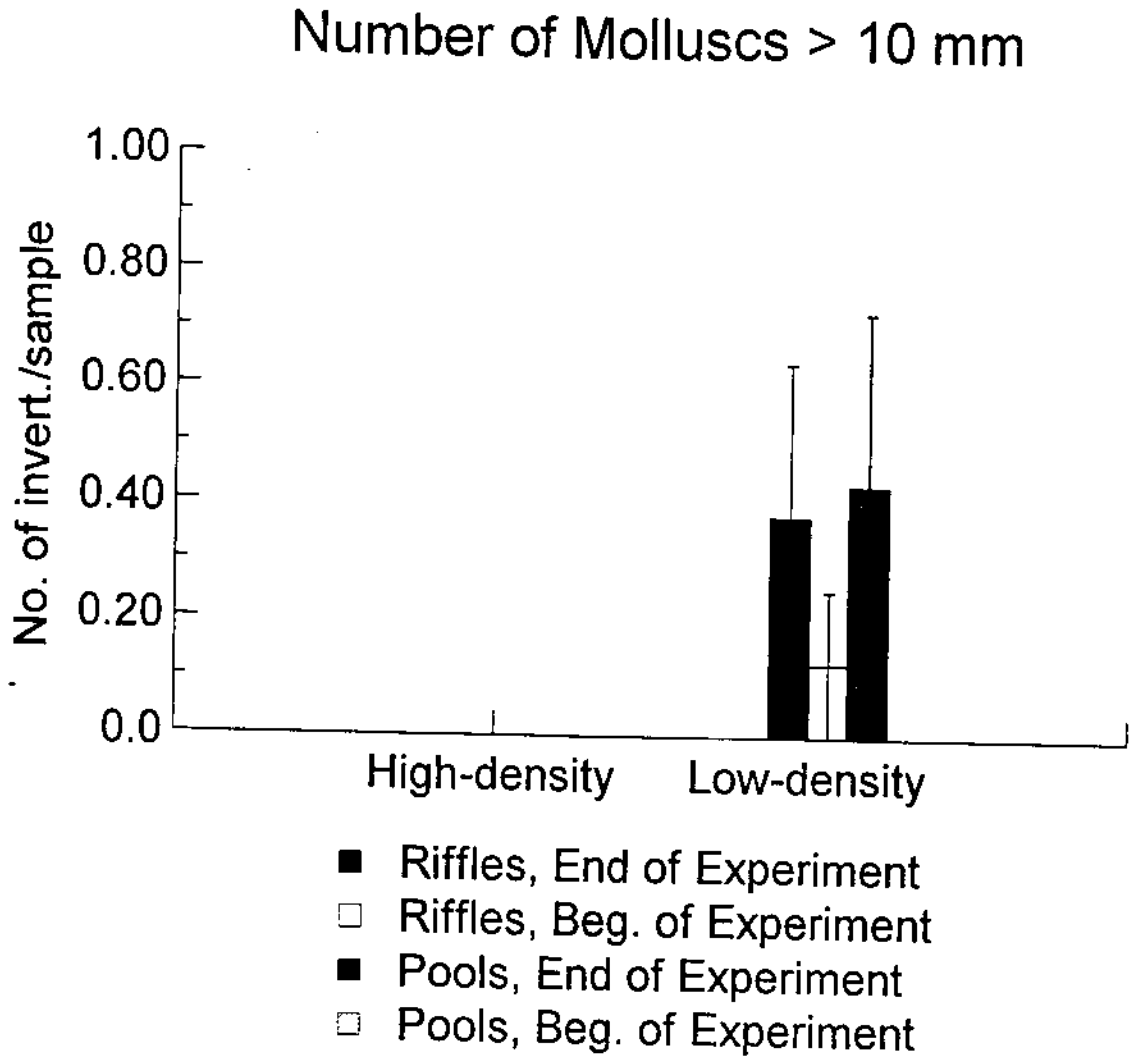


Figure 5. Mean (± SE) abundance of molluscs > 10 mm in Hess samples collected from pools and riffles in sites of high-density and low-density crayfish treatments.

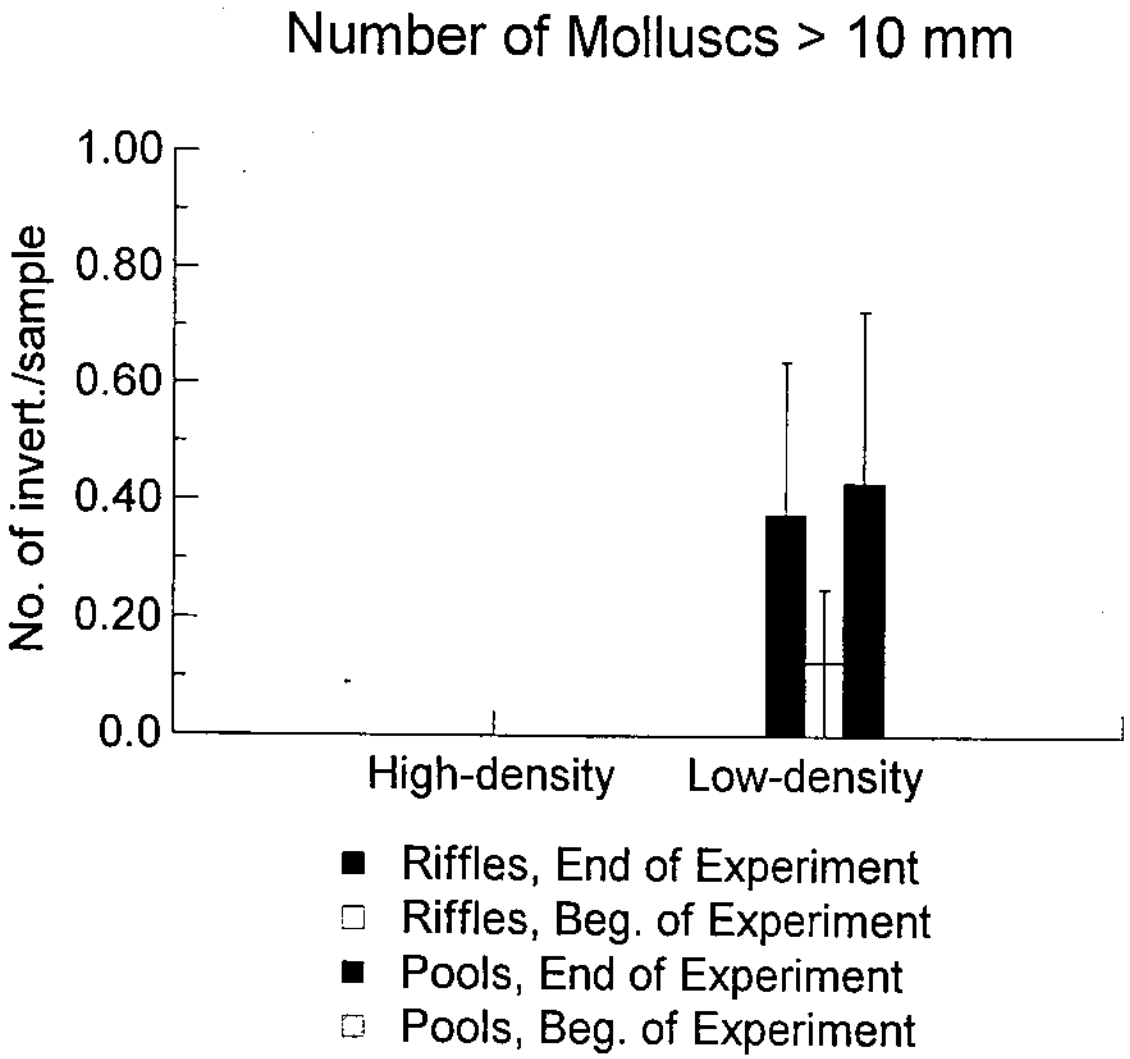


Figure 5. Mean (± SE) abundance of molluscs >10 mm in Hess samples collected from pools and riffles in sites of high-density and low-density crayfish treatments.

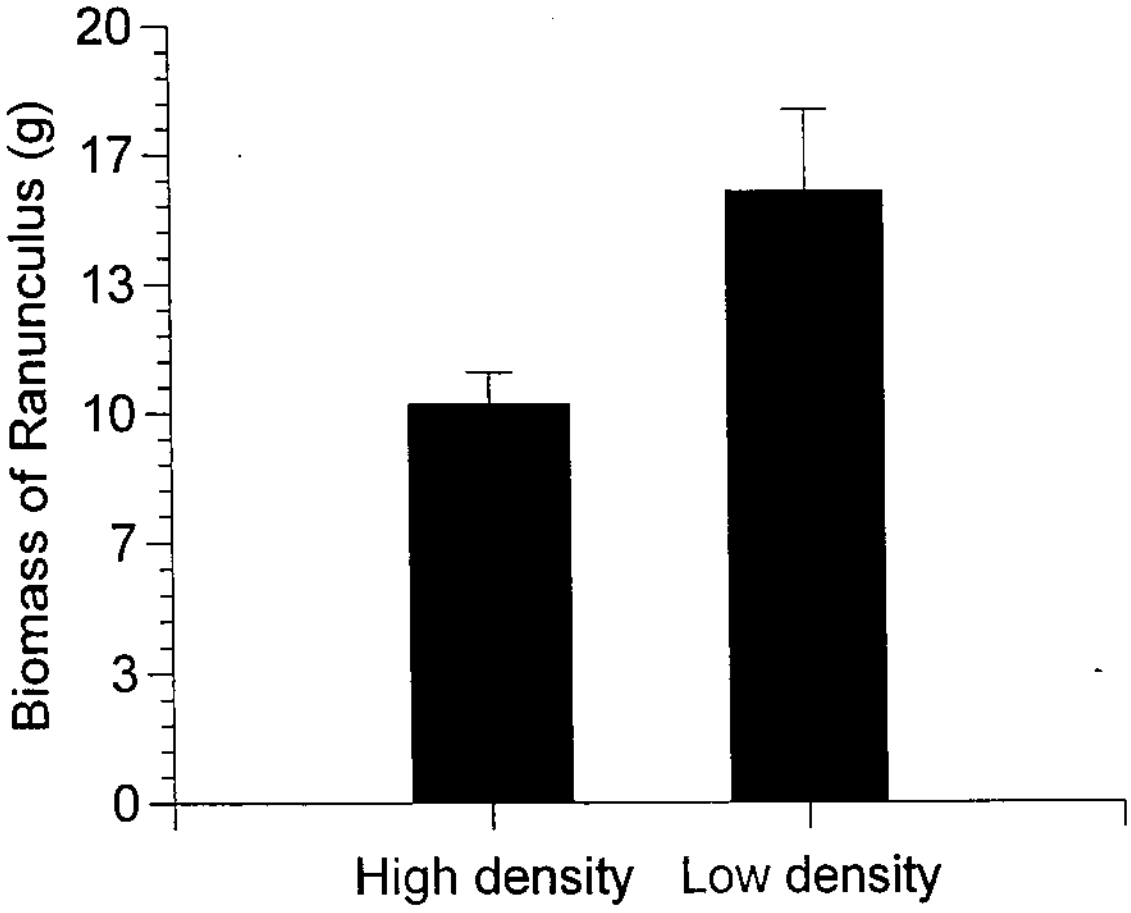


Figure 6. Mean biomass of oven-dried *Ranunculus aquatilis* from high density and low density crayfish treatment sites.

Table 5. Summary of analyses of variance testing effects of crayfish treatment (low or high density) on oven-dried biomass of *Ranunculus aquatilis*. $N = 3$ for all sites except Site 6, where $N = 2$. Site was used as a nesting factor.

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| Biomass of *R. aquatilis* (g) | Trt | 1 | 214.86 | 214.86 | 4.67 | 0.074 |
| | Error(Trt) | 6 | 275.89 | 45.98 | | |

Table 6. Summary of analyses of variance testing effects of crayfish treatment (low or high density), time (pre and post-experiment) and their interaction on benthic macroinvertebrates collected from *Ranunculus aquatilis*.

| Response variable | Factor | df | Sum of Squares | Mean Square | *F* | *P* |
|---|---|---|---|---|---|---|
| Total invertebrate abundance | Trt | 1 | 4281.48 | 4281.48 | 1.25 | 0.307 |
| | Error(Trt) | 6 | 20593.42 | 3432.24 | | |
| Insect diversity (number of orders/sample) | Trt | 1 | 0.08 | 0.08 | 0.05 | 0.831 |
| | Error(Trt) | 6 | 10.05 | 1.67 | | |
| Trichopteran abundance | Trt | 1 | 80.08 | 80.08 | 0.75 | 0.418 |
| | Error(Trt) | 6 | 636.53 | 106.09 | | |
| Mollusc abundance | Trt | 1 | 34.45 | 34.45 | 0.06 | 0.819 |
| | Error(Trt) | 6 | 3598.17 | 599.69 | | |
| Abundance of Molluscs >10 mm | Trt | 1 | 19.59 | 19.59 | 8.66 | 0.026 |
| | Error(Trt) | 6 | 13.58 | 2.26 | | |

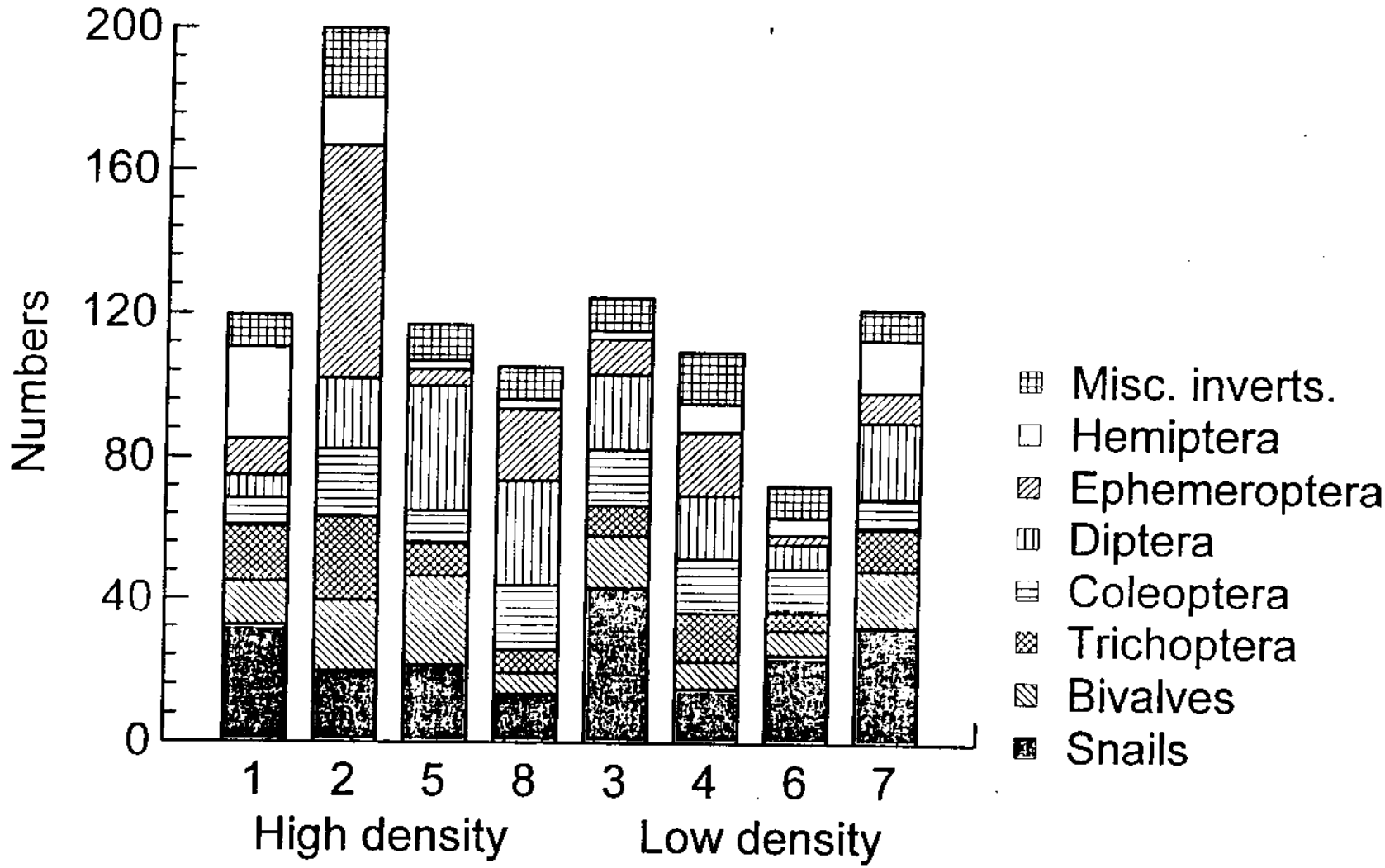


Figure 7. Comparison of numbers of most common aquatic invertebrates collected from samples of *Ranunculus aquatilis*.

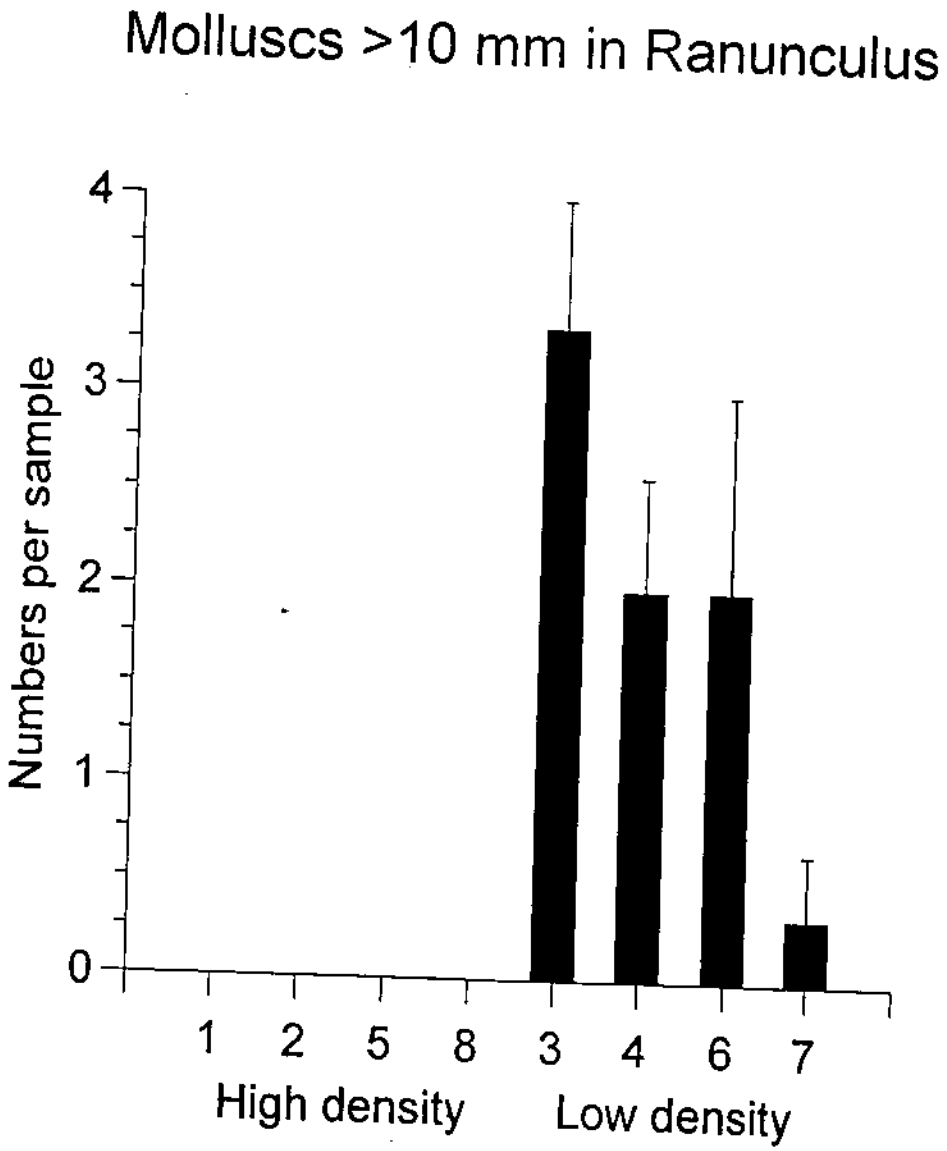


Figure 8. Number of molluscs > 10 mm collected from *Ranunculus aquatilis* samples at end of experiment.

Table 7. Number of fish and crayfish marked and released into field experiment sites.

| Site | Crayfish treatment | Number of fish | Total biomass of fish (g) | Fish density (# / $m^2$) | Number of crayfish | Biomass of crayfish | Crayfish density (# / $m^2$) |
|---|---|---|---|---|---|---|---|
| 1 | High density | 55 | 272.12 | 1.17 | 64 | 2385.70 | 1.36 |
| 2 | High density | 63 | 219.56 | 1.94 | 70 | 2288.55 | 2.16 |
| 3 | Low density | 47 | 256.13 | 2.35 | | | |
| 4 | Low density | 55 | 471.79 | 1.61 | | | |
| 5 | High density | 71 | 674.08 | 1.41 | 91 | 3314.67 | 1.81 |
| 6 | Low density | 53 | 372.96 | 2.23 | | | |
| 7 | Low density | 51 | 375.40 | 1.19 | | | |
| 8 | High density | 40 | 255.15 | 1.23 | 52 | 2137.1 | 1.60 |
| Total | | 435 | | | 277 | | |

decided to make Site 2 a high-density crayfish site, and intensively trapped Site 3 to remove any remaining crayfish. We noted crayfish and fish movement during the experiment by continuously trapping in the sites (Table 8). We trapped most heavily in the low-density crayfish sites because we wanted to avoid fish mortalities. We assumed that mortalities would be higher when setting traps in high-density sites because the crayfish often kill fish in the traps. In addition, we were primarily concerned with keeping crayfish out of low-density sites. Errant crayfish were returned to their assigned site.

Fish response

By 24 September we had marked and measured 435 fish. Our fish densities ranged from 1.19 to 2.35 fish/$m^2$ (Table 7). At the end of the experiment, we captured 470 fish (Table 9). Of these 470 fish, 197 had been previously marked. All the other fish caught were unmarked: of these, 24 were young-of-year and 163 were speckled dace ≤56 mm. We determined that speckled dace ≤56 mm were able to pass through the 1/4-inch mesh of the wiers; therefore we did not use any fish in this category in our analysis. Of the 197 marked fish, we discounted 28 because they were speckled dace ≤ 56 mm, and another 28 because either we could not be sure of their identity or they were not found in their assigned site. Thus for the analysis we had a total sample size of 141 fish. Sites were independent of treatment (Table 10; for Error(Time(Trt)), $P>0.1$), so we could use the full design and thus gain more power in our experiment. However, we found no difference between treatments. For all three species, there was no difference in their weight or condition factor whether they were in high-density sites or low-density sites (Figures 9-11). On average, fish in low-density sites gained more biomass and increased their condition factor more than fish in high-density sites, but differences were not statistically significant (Table 10). Speckled dace increased their biomass and condition factor at all sites, whereas suckers in sites 3, 7, and 8 decreased their biomass and condition factor during the experiment.

Table 8. Results of trapping crayfish and fish to determine amount of movement between sites.

| Site where traps were set | Crayfish treatment | Trapping conducted from 3-24 Oct (during experiment) | | | Trapping and shocking conducted from 9-12 Nov (at end of experiment) | |
|---|---|---|---|---|---|---|
| | | Number of trap-nights | Number of marked crayfish found : Site of origin | Number of marked fish found : Site of origin | Number of marked crayfish found : Site of origin | Number of unmarked crayfish |
| 1 | High density | 10 | 34 : 1<br>1 : 5 | 24 : 1<br>1 : 2 | 12 : 1 | 1 |
| 2 | High density | 2 | 0 | 0 | 9 : 2 | 0 |
| 3 | Low density | 24 | 4 : 2 | 13 : 3 | 1 : 2 | 3 |
| 4 | Low density | 58 | 27 : 5<br>1 : 2 | 5 : 3<br>104 : 4<br>1 : 5 | 1 : 2<br>1 : 5 | 3 |
| 5 | High density | 16 | 18 : 5 | 29 : 5 | 10 : 5 | 0 |
| 6 | Low density | 31 | 4 : 5 | 1 : 4<br>5 : 5<br>25 : 6 | 1 : 5 | 0 |
| 7 | Low density | 40 | 5 : 8 | 14 : 7 | 0 | 0 |
| 8 | High density | 10 | 12 : 8 | 0 | 9 : 8 | 3 |

Table 9. Total number of fish of each species collected at field experiment sites. RHOS = *Rhinichthys osculus*, CAIN = *Catostomus insignis*, CACL = *C. clarkii*. Numbers in parentheses are number of fish from total that were <56 mm.

| Site | Crayfish Treatment | Marked fish, pre-experiment | | | Marked fish, post-experiment | | | Unmarked fish, post-experiment | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | RHOS | CAIN | CACL | RHOS | CAIN | CACL | RHOS | CAIN | CACL | Unid. YOY |
| 1 | High density | 22 (7) | 11 | 23 (3) | 15 (5) | 11 | 4 | 23 (19) | 0 | 9 | 7 |
| 2 | High density | 43 (28) | 7 | 13 | 15 (4) | 5 | 8 | 72 (68) | 3 | 6 | 9 |
| 3 | Low density | 33 (19) | 3 | 11 | 4 (1) | 3 | 1 | 10 (6) | 0 | 4 | 5 |
| 4 | Low density | 28 (14) | 8 | 18 (3) | 20 (6) | 4 | 14 | 18 (17) | 0 | 0 | 2 |
| 5 | High density | 33 (14) | 20 | 18 | 23 (5) | 13 | 9 | 30 (28) | 0 | 1 (1) | 1 |
| 6 | Low density | 35 (19) | 7 | 12 (1) | 6 (1) | 1 | 0 | 2 (0) | 1 | 0 | 0 |
| 7 | Low density | 28 (14) | 12 | 11 | 8 (2) | 9 | 7 | 11 (10) | 0 | 0 | 0 |
| 8 | High density | 22 (6) | 7 | 11 (1) | 13 (4) | 7 | 2 | 47 (14) | 11 | 1 | 0 |

Table 10. Fish response to crayfish treatments. Weight change is the relative change in biomass from beginning to end of experiment, divided by the number of days and multiplied by 44 days (see text for explanation). Change in condition factor is the condition factor at end of experiment - condition factor at beginning of experiment. RHOS = *Rhinicthys osculus*, CAIN = *Catostomus insignis*, CACL = *C. clarkii*.

| Species | Response Variable | High-density sites | | Low-density sites | | ANOVA | | |
|---|---|---|---|---|---|---|---|---|
| | | ($\bar{x}$ ± SE) | *N* | ($\bar{x}$ ± SE) | *N* | df | F-ratio | *P* |
| RHOS | Relative weight change | 4.89 ± 1.06 | 40 | 6.05 ± 0.86 | 23 | 1,61 | 0.57 | 0.453 |
| | Change in condition | 0.066 ± 0.014 | 40 | 0.044 ± 0.01 | 23 | 1,61 | 1.27 | 0.264 |
| CAIN | Relative weight change | 2.72 ± 0.68 | 30 | 1.28 ± 1.10 | 16 | 1,4* | 0.86 | 0.407 |
| | Change in condition | 0.057 ± 0.017 | 30 | 0.026 ± 0.017 | 16 | 1,4* | 0.20 | 0.676 |
| CACL | Relative weight change | 5.18 ± 0.87 | 16 | 8.93 ± 1.99 | 16 | 1,6* | 0.03 | 0.886 |
| | Change in condition | 0.068 ± 0.019 | 16 | 0.057 ± 0.022 | 16 | 1,44 | 1.47 | 0.232 |
| All fish | Change in weight | 0.042 ±0.006 | 86 | 0.055 ± 0.008 | 55 | 1,6* | 0.09 | 0.770 |
| | Change in condition | 0.063 ± 0.009 | 86 | 0.042 ± 0.009 | 55 | 1,6* | 0.30 | 0.604 |

* The degrees of freedom are lower in these cases because there was a significant site effect, so we had to pool all sites.

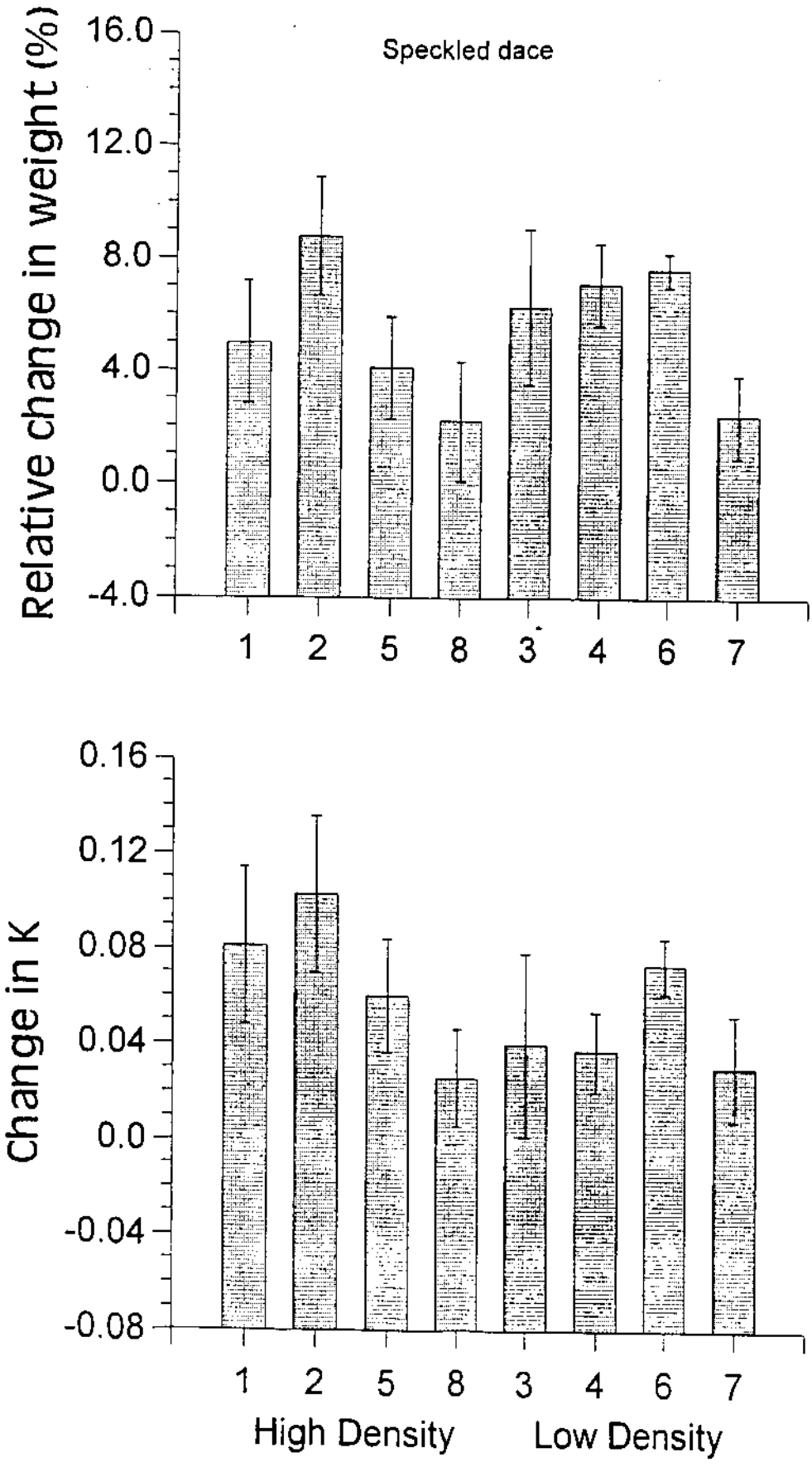


Figure 9. Mean (± SE) change in relative weight (upper graph) and condition factor (lower graph) of speckled dace during the field experiment.

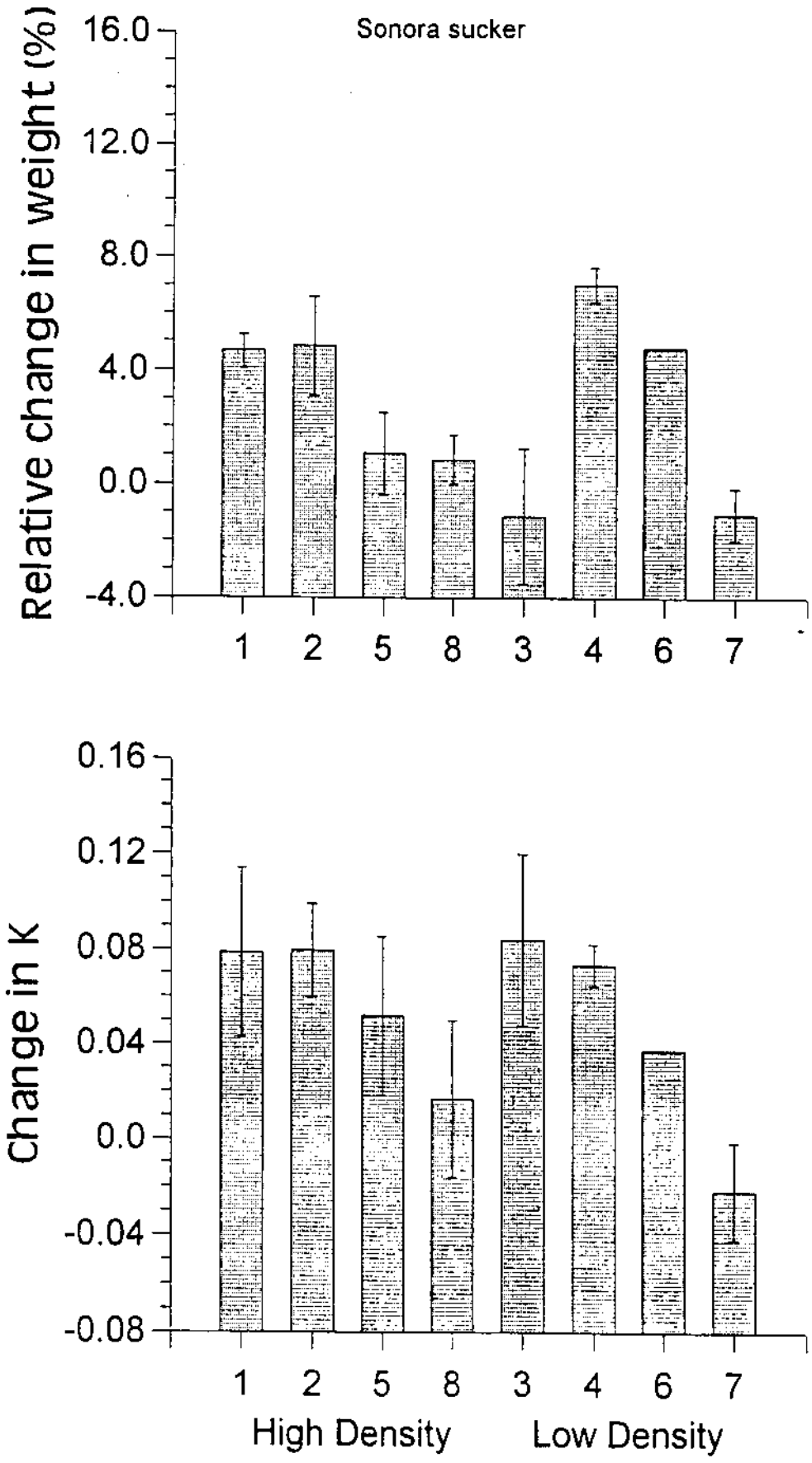


Figure 10. Mean (± SE) change in relative weight (upper graph) and condition factor (lower graph) of Sonora sucker during the field experiment.

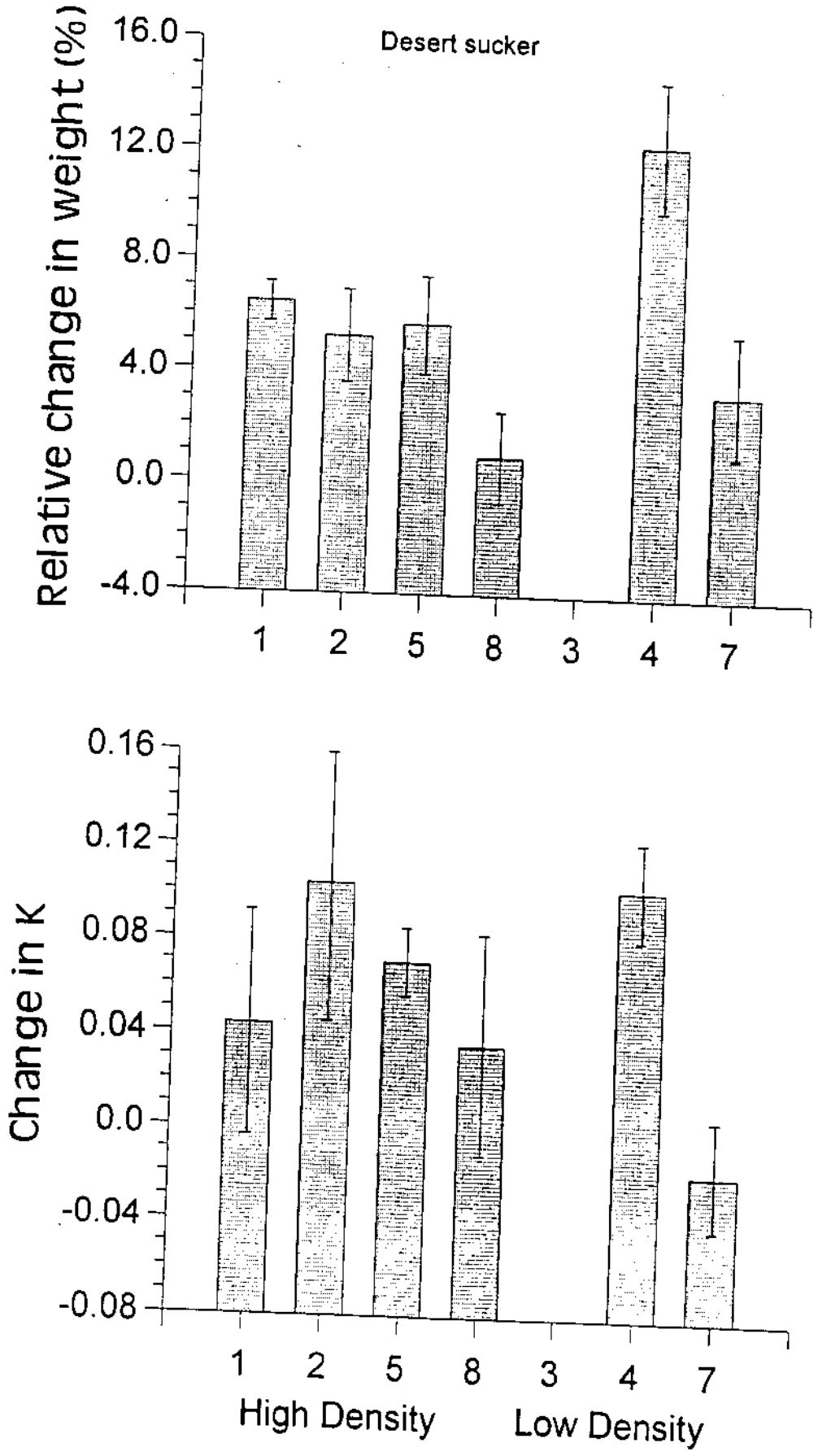


Figure 11. Mean (± SE) change in relative weight (upper graph) and condition factor (lower graph) of desert sucker during the field experiment.

## DISCUSSION

We saw no significant change in fish response to crayfish density; therefore, we can not reject the null hypothesis that crayfish do not compete for forage with the three fish species used in this experiment. Wiens (1977) suggests that competition is often an intermittent process, and it may be difficult to see clear evidence of competition in short-term field studies unless communities are in resource-definedequilibrium (i.e., the populations are at carrying capacity and thus the habitats are saturated). Like many other researchers examining competition, we assumed that crayfish and native fish populations at Three Forks were at carrying capacity. This assumption may not be valid.

Our experiment was of short duration: we measured changes in weight, length, and condition factor over at least 44 days to evaluate competition between crayfish and fish, and we measured macroinvertebrate and *Ranunculus aquatilis* response to treatment over at least 38 days. Many other researchers have successfully observed significant changes in fish and crayfish response during short-duration experiments (for examples see discussion in Part I of this report). Changes in invertebrate abundance and macrophyte biomass have been examined in numerous short-term studies of less than 40 days (e.g., Hanson et al. 1990, 35 days; Holomuszki et al. 1994, 23 days; Blois-Heulin et al. 1990, 30 days; Lodge and Lorman 1987; 35 days). Thus it seems likely that significant changes in macroinvertebrate abundance can be obtained from short-term studies.

We found the effects of crayfish on *Ranunculus aquatilis* and associated macroinvertebrates were similar to the results of Rosen and Fernandez (1996) for the same stream. We observed lower biomass of *R. aquatilis* and virtually no large (>10 mm) molluscs in high-density crayfish sites compared to low-density crayfish sites. However, our benthic macroinvertebrate data obtained with a modified Hess sampler indicated differences in insect diversity and abundance of trichopterans and large molluscs, yet these differences were present before the experiment began. Thus it is difficult to compare our benthic invertebrate data with the field data of Rosen and Fernandez (1996). They found that mean number of benthic macroinvertebrates was significantly lower in sites with no crayfish compared to sites with crayfish, and found significant declines with specific taxa: trichopteran larvae, snails, and the mussel *Anodonta californiensis*. Their lab work supported what they observed in the field: trichopteran larvae and snails were nearly completely eliminated from tanks with crayfish. There are several explanations for our invertebrate data not closely matching those of Rosen and Fernandez (1996). One explanation is that we set up the field experiment in a stretch of stream that had supported a substantial crayfish population. Therefore, the invertebrate population had already

been impacted by crayfish before the start of the experiment. In addition, our treatments differed only in the relative abundance of crayfish, not their total absence from half the sites. Finally, our study was conducted in autumn whereas Rosen and Fernandez (1996) was conducted in summer. Crayfish metabolic activity and feeding were undoubtedly lower during our study because of lower temperatures.

Various researchers have examined the effect of crayfish on stream invertebrates and vegetation. During a 46-day experiment, Charlebois and Lamberti (1996) found that a non-native crayfish (at densities of 5-10 crayfish/$m^2$) in enclosures significantly lowered total invertebrate as well as herbivorous invertebrate densities compared to unenclosed areas. They also determined that taxonomic richness declined, but periphyton increased, in the presence of crayfish. Hanson et al. (1990) determined that snail abundance was greatly reduced in laboratory pools with high-crayfish densities compared to pools where crayfish were absent. The results of these studies are similar to what we found at Three Forks. Charlebois and Lamberti (1996) suggest that crayfish reduce invertebrates not only by direct consumption but also by increasing drift due to disturbance; and possibly by inhibiting colonization. Hoekstra (1998) compared macroinvertebrate communities in streams with and without crayfish, and observed different effects of crayfish depending on stream habitat: in pools, there was a negative relationship between crayfish presence and taxon richness; in riffles, there was a positive relationship between crayfish presence and specific collector-gatherer and algivorous invertebrate taxa.

Site 3 was significantly shallower than other sites (Table 1), and during the cold spell that occurred during this experiment, freezing conditions may have limited habitat even more. Fish in this site may have experienced harsher conditions than at other sites; this factor may explain why Sonora sucker had such low weights and condition factor in this site (Figure 10), and why desert sucker were missing (Figure 11). However, we re-analyzed the fish data without Site 3; the results did not change, and Site 3 was not entirely responsible for the significant interaction between sites and treatments.

We could not prevent movement of all fish and crayfish between sites. Movement may have compromised our experimental design and limited our interpretation of the results. However, the amount of movement between sites was minor or very short-term (as in the case of crayfish in Site 3 moving to Site 2, or crayfish in Site 5 moving into Site 4). Therefore, we do not think movement of experimental animals significantly affected our results.

The experiment was conducted during the autumn when water temperatures were relatively low. Therefore our results may be conservative. Fish and crayfish are poikilothermic, and generally fish feed less actively at cool temperatures. Futhermore, declining photoperiod may affect predation rates (Wootton 1992). The rate at which *O. virilis* fed on trout eggs decreased with temperature, but feeding continued at temperatures as low as 2°C (Horns and Magnuson 1981).

At the end of the experiment, our re-capture rates of fish and crayfish were low; we suspect the low capture rate was due to inactivity at low temperatures rather than missing animals. *Orconectes virilis* in Nutria Lake, New Mexico (<200 km from Three Forks) stop growing in late September to early October, but only a small percentage of adult crayfish hibernate, and some remain active all winter (Dean 1969). Likewise, we noted that when stream temperatures were at their minimum, we still saw active crayfish.

Runck and Blinn (1993) suggest that fish in White Mountain streams may also remain fairly active during winter. They examined the seasonal diet of Little Colorado spinedace in Nutrioso Creek (<20 km from Three Forks) and determined that stomach fullness in October was not different from that in May and July. Further, the percentage of empty stomachs in October was not different from that in July. Although stomach samples were not very different in autumn and summer, density of benthic invertebrates decreased: Surber sample estimates averaged 3,870 insects/$m^2$ in July and 249 insects/$m^2$ in October. These data suggest that the amount of benthic forage was reduced in October but that fish continued to feed successfully. These results support our assumption that native fish in Three Forks continued to feed despite cooler water temperatures and possibly decreased benthic resources.

These results suggest that fish of the White Mountains may remain active under lower temperature conditions than what would be expected of the same speies in desert habitats. Clearly it is advantageous for fish at high altitudes to remain active at lower temperatures than the same species at lower altitudes.

High densities of crayfish reduce *Ranunculus aquatilis* biomass and eliminate the larger (> 10 mm) molluscs associated with this aquatic plant. High densities of crayfish also reduced diversity of benthic insects. There was no clear effect of crayfish on biomass and condition of speckled dace, Sonora sucker, or desert sucker. Explanations for the lack of effect on fishes include the following: there is no competition for forage between the three fish and *O. virilis*; populations of fish and crayfish were not at carrying capacity or food was not limiting during the study; competition exists, but was not observed due to the short study period or the cool temperatures present when the study

was conducted; fish and crayfish moved between pools thereby blurring the differences between treatments.

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Appendix A-1. Numbers of invertebrates collected from riffles using modified Hess sampler, pre- experiment.

| | Sites with high densities of crayfish | | | | | | | | Sites with low densities of crayfish | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 1 | 2 | 2 | 5 | 5 | 8 | 8 | 3 | 3 | 4 | 4 | 6 | 6 | 7 | 7 |
| Station | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Mean depth (cm) | 4 | 8 | 7 | 6 | 10 | 8 | 4 | 10 | 9 | 6 | 19 | 7 | 5 | 5 | 8 | 8 |
| Acarina | 0 | 3 | 5 | 4 | 0 | 0 | 0 | 0 | 1 | 11 | 0 | 0 | 1 | 1 | 0 | 0 |
| Arachnida | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Crayfish | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Amphipoda | 1 | 2 | 0 | 3 | 5 | 8 | 4 | 11 | 0 | 0 | 4 | 4 | 9 | 6 | 8 | 5 |
| Ostracoda | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1 | 0 |
| Oligochaete | 28 | 38 | 4 | 4 | 3 | 11 | 11 | 55 | 36 | 52 | 32 | 0 | 0 | 1 | 140 | 170 |
| Hirudinea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gastropoda | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 2 | 0 | 0 | 0 |
| Bivalvia | 2 | 22 | 0 | 3 | 0 | 0 | 6 | 0 | 10 | 1 | 0 | 15 | 9 | 0 | 0 | 1 |
| Coleoptera | 45 | 38 | 199 | 110 | 5 | 5 | 50 | 0 | 9 | 73 | 19 | 110 | 232 | 202 | 1 | 12 |
| Diptera | 83 | 111 | 103 | 116 | 137 | 95 | 615 | 25 | 267 | 337 | 395 | 166 | 301 | 701 | 73 | 67 |
| Ephemeroptera | 104 | 485 | 246 | 155 | 22 | 16 | 105 | 3 | 57 | 288 | 105 | 129 | 64 | 108 | 10 | 62 |
| Hemiptera | 11 | 12 | 24 | 5 | 1 | 6 | 5 | 7 | 14 | 13 | 6 | 11 | 4 | 6 | 38 | 1 |
| Homoptera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lepidoptera | 2 | 2 | 32 | 5 | 0 | 2 | 0 | 0 | 0 | 4 | 2 | 7 | 2 | 4 | 0 | 0 |
| Odonata | 0 | 2 | 3 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Plecoptera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trichoptera | 7 | 7 | 55 | 21 | 2 | 5 | 26 | 0 | 7 | 9 | 1 | 42 | 34 | 77 | 8 | 0 |
| Total abundance | 283 | 723 | 673 | 428 | 175 | 150 | 823 | 103 | 403 | 790 | 569 | 486 | 658 | 1106 | 281 | 319 |
| Abundance of insects and molluscs | 254 | 680 | 663 | 417 | 167 | 129 | 808 | 35 | 365 | 727 | 529 | 482 | 648 | 1098 | 130 | 144 |
| Insect diversity | 6 | 7 | 7 | 7 | 5 | 6 | 6 | 3 | 5 | 7 | 7 | 6 | 6 | 6 | 5 | 5 |
| Molluscs 0-4 mm | 2 | 22 | 0 | 3 | 0 | 0 | 6 | 0 | 10 | 1 | 0 | 16 | 9 | 0 | 0 | 1 |
| Molluscs 5-10 mm | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| Molluscs >10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Appendix A-2 . Numbers of invertebrates collected from pools using modified Hess sampler, pre- experiment.

| | Sites with high densities of crayfish | | | | | | | | Sites with low densities of crayfish | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 1 | 2 | 2 | 5 | 5 | 8 | 8 | 3 | 3 | 4 | 4 | 6 | 6 | 7 | 7 |
| Station | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Mean depth (cm) | 29 | 35 | 30 | 36 | 39 | 35 | 25 | 32 | 14 | 35 | 35 | 17 | 34 | 13 | 14 | 10 |
| Acarina | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Arachnida | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Crayfish | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 |
| Amphipoda | 0 | 2 | 0 | 0 | 7 | 0 | 0 | 2 | 5 | 3 | 1 | 45 | 3 | 13 | 4 | 2 |
| Ostracoda | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oligochaete | 38 | 300 | 9 | 28 | 5 | 1 | 18 | 10 | 145 | 297 | 10 | 5 | 64 | 41 | 74 | 52 |
| Hirudinea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Gastropoda | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 |
| Bivalvia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Coleoptera | 1 | 9 | 0 | 4 | 0 | 0 | 2 | 0 | 6 | 5 | 6 | 6 | 2 | 14 | 3 | 1 |
| Diptera | 6 | 40 | 1 | 20 | 1 | 1 | 15 | 1 | 257 | 98 | 17 | 57 | 15 | 116 | 31 | 24 |
| Ephemeroptera | 4 | 6 | 0 | 1 | 1 | 0 | 1 | 0 | 87 | 64 | 4 | 8 | 7 | 9 | 8 | 3 |
| Hemiptera | 44 | 12 | 1 | 0 | 3 | 1 | 0 | 8 | 1 | 0 | 0 | 2 | 9 | 3 | 72 | 1 |
| Homoptera | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Lepidoptera | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Odonata | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Plecoptera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trichoptera | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 0 | 7 | 0 | 6 | 0 | 2 |
| Total abundance | 94 | 371 | 11 | 56 | 17 | 3 | 37 | 22 | 506 | 471 | 38 | 138 | 100 | 202 | 192 | 87 |
| Abundance of insects and molluscs | 56 | 68 | 2 | 54 | 5 | 2 | 19 | 10 | 353 | 171 | 27 | 85 | 33 | 148 | 114 | 32 |
| Insect diversity | 5 | 5 | 2 | 3 | 3 | 2 | 4 | 3 | 5 | 5 | 3 | 7 | 4 | 5 | 4 | 5 |
| Molluscs 0-4 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Molluscs 5-10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Molluscs >10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appendix A-3. Numbers of invertebrates collected from riffles using modified Hess sampler, post- experiment.

| | Sites with high densities of crayfish | | | | | | | Sites with low densities of crayfish | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 1 | 2 | 2 | 5 | 8 | 8 | 3 | 3 | 4 | 4 | 6 | 7 | 7 |
| Station | 1 | 2 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 2 |
| Mean depth (cm) | 26 | 10 | 10 | 12 | 12 | 10 | 11 | 9 | 11 | 21 | 10 | 14 | 19 | 15 |
| Acarina | 1 | 1 | 2 | 7 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 0 |
| Arachnida | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Crayfish | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amphipoda | 2 | 2 | 0 | 33 | 19 | 33 | 25 | 0 | 0 | 7 | 4 | 22 | 13 | 4 |
| Ostracoda | 3 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Oligochaete | 69 | 8 | 1 | 67 | 18 | 14 | 5 | 1 | 7 | 46 | 0 | 13 | 60 | 7 |
| Hirudinea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 |
| Gastropoda | 0 | 0 | 0 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 2 | 1 | 2 | 0 |
| Bivalvia | 1 | 15 | 0 | 4 | 0 | 1 | 4 | 0 | 0 | 0 | 12 | 2 | 0 | 11 |
| Coleoptera | 6 | 31 | 109 | 56 | 5 | 1 | 5 | 77 | 66 | 16 | 134 | 19 | 3 | 29 |
| Diptera | 204 | 87 | 33 | 272 | 120 | 265 | 188 | 220 | 177 | 608 | 224 | 133 | 57 | 169 |
| Ephemeroptera | 15 | 154 | 180 | 109 | 9 | 31 | 16 | 265 | 220 | 33 | 326 | 68 | 20 | 75 |
| Hemiptera | 1 | 17 | 3 | 7 | 4 | 0 | 2 | 5 | 25 | 0 | 8 | 0 | 3 | 0 |
| Homoptera | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Lepidoptera | 0 | 4 | 20 | 2 | 0 | 1 | 0 | 13 | 24 | 0 | 23 | 1 | 0 | 0 |
| Odonata | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 1 | 1 | 0 | 4 | 0 | 3 | 1 |
| Plecoptera | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trichoptera | 2 | 8 | 35 | 13 | 10 | 7 | 3 | 17 | 34 | 2 | 74 | 5 | 5 | 2 |
| Total abundance | 305 | 328 | 383 | 577 | 186 | 354 | 250 | 603 | 555 | 716 | 811 | 266 | 166 | 302 |
| Abundance of insects and molluscs | 230 | 317 | 380 | 469 | 149 | 306 | 219 | 599 | 548 | 659 | 807 | 229 | 93 | 289 |
| Insect diversity | 6 | 7 | 6 | 8 | 6 | 5 | 5 | 7 | 7 | 4 | 7 | 5 | 6 | 6 |
| Molluscs 0-4 mm | 1 | 15 | 0 | 5 | 0 | 1 | 5 | 1 | 0 | 0 | 12 | 2 | 0 | 11 |
| Molluscs 5-10 mm | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 7 | 0 | 2 | 0 | 0 | 0 |
| Molluscs >10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 |

Appendix A-4. Numbers of invertebrates collected from pools using modified Hess sampler, post- experiment.

| | Sites with high densities of crayfish | | | | | | | | Sites with low densities of crayfish | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 1 | 2 | 2 | 5 | 5 | 8 | 8 | 3 | 3 | 4 | 4 | 6 | 6 | 7 | 7 |
| Station | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Mean depth (cm) | 34 | 36 | 39 | 39 | 42 | 40 | 51 | 50 | 13 | 20 | 37 | 39 | 34 | 36 | 46 | 40 |
| Acarina | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 0 | 1 | 0 | 0 | 0 | 2 | 0 |
| Arachnida | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Crayfish | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| Amphipoda | 2 | 0 | 4 | 0 | 1 | 0 | 12 | 0 | 1 | 18 | 0 | 4 | 1 | 8 | 2 | 0 |
| Ostracoda | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oligochaete | 9 | 22 | 10 | 0 | 184 | 56 | 188 | 209 | 4 | 111 | 13 | 99 | 45 | 22 | 141 | 44 |
| Hirudinea | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 |
| Gastropoda | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 3 | 0 | 0 | 0 | 1 | 1 | 0 |
| Bivalvia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Coleoptera | 1 | 5 | 2 | 1 | 27 | 0 | 1 | 4 | 24 | 21 | 6 | 14 | 12 | 8 | 0 | 9 |
| Diptera | 38 | 76 | 9 | 75 | 48 | 3 | 99 | 61 | 101 | 731 | 69 | 154 | 73 | 20 | 20 | 9 |
| Ephemeroptera | 5 | 0 | 4 | 0 | 4 | 0 | 11 | 6 | 281 | 105 | 2 | 8 | 4 | 2 | 5 | 2 |
| Hemiptera | 0 | 48 | 3 | 1 | 4 | 0 | 0 | 4 | 10 | 3 | 4 | 28 | 3 | 5 | 8 | 57 |
| Homoptera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Lepidoptera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| Odonata | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Plecoptera | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trichoptera | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 12 | 7 | 1 | 2 | 6 | 2 | 2 | 0 |
| Total abundance | 55 | 151 | 32 | 78 | 269 | 60 | 317 | 287 | 473 | 1002 | 97 | 310 | 147 | 72 | 181 | 121 |
| Abundance of insects and molluscs | 44 | 129 | 18 | 77 | 83 | 3 | 115 | 77 | 463 | 873 | 83 | 206 | 98 | 40 | 36 | 77 |
| Insect diversity | 3 | 3 | 4 | 3 | 4 | 1 | 4 | 5 | 6 | 7 | 6 | 5 | 5 | 6 | 4 | 4 |
| Molluscs 0-4 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 7 | 2 | 0 | 0 | 0 | 1 | 1 | 0 |
| Molluscs 5-10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Molluscs >10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

Appendix B. Numbers of invertebrates collected from samples of *Ranunculus aquatilis*.

| | Sites with high densities of crayfish | | | | | | | | | | | | | | Sites with low densities of crayfish | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Site 1 | | Site 2 | | | Site 5 | | | Site 8 | | | Site 3 | | | Site 4 | | | Site 6 | | Site 7 | | |
| Order/Class | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1 | 2 | 3 |
| Acarina | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| Arachnida | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Crayfish | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amphipoda | 167 | 87 | 99 | 65 | 103 | 86 | 52 | 110 | 58 | 115 | 108 | 5 | 101 | 57 | 115 | 250 | 147 | 118 | 98 | 49 | 41 | 62 |
| Ostracoda | 9 | 5 | 8 | 28 | 1 | 3 | 0 | 6 | 0 | 5 | 27 | 2 | 4 | 4 | 9 | 7 | 8 | 2 | 5 | 55 | 7 | 8 |
| Oligochaete | 0 | 1 | 3 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hirudinea | 8 | 2 | 4 | 6 | 1 | 6 | 4 | 4 | 6 | 5 | 10 | 9 | 5 | 3 | 8 | 3 | 9 | 5 | 6 | 6 | 7 | 4 |
| Gastopoda | 26 | 39 | 9 | 35 | 15 | 25 | 6 | 33 | 2 | 13 | 25 | 46 | 33 | 51 | 20 | 8 | 17 | 19 | 30 | 24 | 42 | 31 |
| Bivalvia | 12 | 13 | 10 | 48 | 2 | 43 | 1 | 32 | 0 | 3 | 15 | 21 | 10 | 13 | 0 | 10 | 13 | 5 | 9 | 27 | 15 | 7 |
| Coleoptera | 1 | 15 | 32 | 12 | 13 | 7 | 13 | 8 | 11 | 27 | 17 | 23 | 11 | 13 | 7 | 13 | 26 | 10 | 15 | 12 | 5 | 9 |
| Diptera | 2 | 11 | 20 | 31 | 8 | 44 | 23 | 38 | 14 | 5 | 70 | 8 | 32 | 23 | 9 | 21 | 24 | 7 | 7 | 15 | 21 | 28 |
| Ephemeroptera | 1 | 19 | 69 | 75 | 51 | 6 | 4 | 4 | 0 | 2 | 58 | 5 | 18 | 7 | 3 | 28 | 22 | 1 | 4 | 16 | 6 | 3 |
| Hemiptera | 30 | 21 | 6 | 31 | 4 | 1 | 1 | 5 | 1 | 2 | 5 | 0 | 6 | 1 | 12 | 6 | 6 | 2 | 8 | 10 | 12 | 22 |
| Homoptera | 0 | 1 | 1 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lepidoptera | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 1 |
| Odonata | 2 | 3 | 0 | 9 | 6 | 5 | 2 | 9 | 0 | 3 | 2 | 1 | 3 | 0 | 2 | 8 | 7 | 2 | 5 | 1 | 2 | 0 |
| Trichoptera | 12 | 19 | 16 | 33 | 22 | 8 | 6 | 13 | 0 | 4 | 15 | 8 | 15 | 3 | 5 | 23 | 13 | 7 | 3 | 10 | 16 | 9 |
| Total Abundance | 270 | 237 | 277 | 394 | 233 | 234 | 112 | 263 | 92 | 184 | 353 | 130 | 242 | 175 | 190 | 381 | 294 | 178 | 190 | 226 | 175 | 186 |
| Abundance of macroinvertebrates | 94 | 145 | 170 | 301 | 129 | 145 | 60 | 146 | 34 | 64 | 218 | 123 | 137 | 114 | 66 | 124 | 139 | 58 | 87 | 122 | 127 | 116 |
| Insect Diversity | 6 | 7 | 6 | 8 | 8 | 6 | 6 | 6 | 3 | 6 | 6 | 6 | 6 | 5 | 6 | 7 | 7 | 6 | 6 | 6 | 6 | 6 |
| Molluscs <4 mm | 23 | 25 | 16 | 53 | 10 | 55 | 3 | 39 | 2 | 8 | 24 | 52 | 7 | 22 | 6 | 7 | 19 | 11 | 17 | 32 | 16 | 24 |
| Molluscs 5-10 mm | 15 | 27 | 3 | 30 | 7 | 13 | 4 | 26 | 0 | 8 | 16 | 15 | 28 | 40 | 11 | 9 | 10 | 10 | 21 | 19 | 41 | 13 |
| Molluscs > 10 mm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 2 | 3 | 2 | 1 | 3 | 1 | 0 | 0 | 1 |

# COMPETITION AND PREDATION IN A LABORATORY SETTING BETWEEN AN INTRODUCED CRAYFISH (*ORCONECTES VIRILIS*) AND NATIVE FISHES OF ARIZONA

Jeanette Carpenter

and

Carole McIvor

Arizona Cooperative Fish and Wildlife Research Unit
Bio Sciences East #104, University of Arizona, Tucson, AZ 85721

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# Competition and Predation in a Laboratory Setting Between an Introduced Crayfish (*Orconectes virilis*) and Native Fishes of Arizona

## CONTENTS



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## ABSTRACT

We conducted laboratory experiments to examine the level of predation and competition for shelter between the non-native crayfish (*Orconectes virilis*) and three native Arizona fishes: Gila chub (*Gila intermedia*), desert sucker (*Catostomus clarki*), and speckled dace (*Rhinichthys osculus*). For the competition experiments, we used a crayfish that was of equal or smaller size than the three native fish. We used green sunfish (*Lepomis cyanellus*) as a predator to elicit a stronger response from both crayfish and native fish in seeking shelter. Crayfish displaced native fish from shelter and attacked them several times. None of the native fish attacked the crayfish, and out of 19 trials, only one native fish (a desert sucker) displaced a crayfish. Although native fish sought cover during control trials and when green sunfish were visible through a clear partition, they never used shelter for refuge when the partition was removed.

We evaluated vulnerability of Gila chub and desert sucker to predation by large crayfish (>3.5 cm carapace length). Crayfish preyed upon both fish species; however crayfish preyed more heavily upon desert suckers than on Gila chub. It is likely that desert suckers were more vulnerable because they used primarily the lower portion of the water column, whereas Gila chub used the entire water column. Neither native fish species altered their use of the water column in the presence of crayfish. This lack of a behavioral response to a predator demonstrates "naivety" and likely derives from the lack of a common evolutionary history.

## INTRODUCTION

Crayfish have long been considered primarily herbivores and detritivores (Momot et al. 1978). However, recent work demonstrates that many crayfish species actively prey on live animals (Crowl 1989; Hobbs et al. 1989; Hanson et al. 1990; Axelsson et al. 1997). Momot (1995) asserts that crayfish can be the chief carnivore in many streams. Much work has focused on crayfish as prey for fish (e.g., Stein and Magnuson 1976; Mather and Stein 1993; Garvey et al. 1994). Few papers have examined the role of crayfish as fish predators (Minckley and Craddock 1961; Matity et al.1994; Guan and Wiles 1997). Numerous authors have reported observing crayfish predation upon fish (see Hobbs 1993 for extensive review). Minckley and Craddock (1961) described crayfish actively preying upon fish that were incapacitated by electroshock or rotenone. Crayfish may actively feed on fish

eggs in laboratory (e.g., Horns and Magnuson 1981; Lodge et al. 1985; Miller et al. 1992) or field (White 1995) settings.

Crayfish often seek cover in the presence of predatory fish, and crayfish can outcompete small fish for refuge. Guan and Wiles (1997) determined that crayfish from a British lowland river immediately evicted small (≤105 mm TL) benthic fish from shelters in an artificial stream and noted the benthic fish significantly reduced their use of shelter in the presence of crayfish. Rahel and Stein (1988) reported that crayfish evicted johnny darters (*Etheostoma nigrum*) from shelters in the presence of smallmouth bass (*Micropterus dolomieui*), thus increasing predation on this benthic fish. In contrast, McNeely et al. (1990) noted that mottled sculpin (*Cottus bairdi*) increased their use of cover and decreased their vulnerability to predation by smallmouth bass when in the presence of crayfish.

*Orconectes virilis* is a non-native crayfish that has become widely distributed in Arizona streams (Inman et al. 1998). Despite its broad distribution, little research has been conducted on the effect of this species on aquatic ecosystems in Arizona (Dean 1969; Fernandez and Rosen 1996; Carpenter and McIvor 1998). Non-native crayfish may alter stream community structure due to their polytrophic habits, aggressive nature, and ability to alter habitats (Hobbs et al. 1989; Momot 1995). Therefore, it is important to determine the impact of *O. virilis* on native Arizona fish.

Documenting predation on fish by crayfish can be difficult under field conditions. For instance, crayfish stomachs could be analyzed for fish remains. However there would be no way to determine if fish were captured live or merely scavenged. Laboratory experiments, while often artificial in terms of habitat simulation, allow researchers to clearly quantify species interactions. Therefore we chose to examine the potential for *O. virilis* to actively prey upon and compete for shelter with native stream fish in laboratory experiments. The results of this study should help in corroborating the results of our recent field work.

We focused on Gila chub (*Gila intermedia*), desert sucker (*Catostomus clarki*), and speckled dace (*Rhinichthys osculus*). Our objectives were to determine if *O. virilis* competes with native fish for refuge from predation, and if *O. virilis* preys differentially on different fish species. For refuge competition experiments, we used green sunfish (*Lepomis cyanellus*) as the predator. Green sunfish is a non-native fish found in many Arizona streams, and a known predator on all three fish species (Dudley 1995; personal observations) as well as on crayfish (Hobbs 1993).

# METHODS AND MATERIALS

## General laboratory setup

Native fish and green sunfish were held in separate 113-L aquaria and fed dried fish flakes, sinking shrimp pellets, and frozen brine shrimp ad libitum. Crayfish were kept in a plastic tray (34 cm wide X 51 cm long X 21 cm high) and fed shrimp and rabbit food pellets ad libitum. To prevent experimental animals from being disturbed by observers, we covered test aquaria with auto tint film on the outer pane (Chick and McIvor 1997), and black plastic or black paper on the remaining three panes. Outer panes of the experimental aquaria were marked at the 7.5, 15, and 22.5 cm level; these lines denoted the lower, middle, and upper third of the water column, respectively. All experiments were run with water at the 22.5-cm level. The laboratory was lighted with fluorescent bulbs on a 12-h light, 12-h dark cycle.

## Experiment I: Competition for shelter in presence of predatory fish

The purpose of this experiment was to determine if crayfish compete with native fish for refuge. We used 2 bricks set in the substrate vertically to create a refuge area between them that was 2.5 cm wide and 8 cm deep. Native fish and crayfish could enter this protected area from any portion of the water column. Our goal was to create a refuge similar to interstitial spaces found in nature. The refuge space was large enough for one individual on the bottom, but narrow and deep enough to prevent green sunfish from entering the shelter or from removing prey. We added a green sunfish, a potential predator of crayfish and native fish, to encourage interactions between the native fish and crayfish for the single shelter in the tank. Four green sunfish were used over the course of the experiments. They ranged in size from 17-19 cm TL. A 2-part partition--one clear plexiglass and the other opaque (both with pores)--divided the tank in half, with the refuge on one side. A single green sunfish was placed on the side of the tank without shelter and allowed to acclimate for several days. The similarly-sized prey (one native fish and one crayfish) were placed on the side of the tank with the shelter. This set-up represented the control part of the experiment. We assumed that the animals could sense each other, but the green sunfish could not see the crayfish and native fish. For the next 1.25 h, we recorded interactions between the native fish and crayfish, including number of attacks and

displacements from the shelter. We also recorded the frequency with which each animal used the shelter. The opaque divider was then removed, thus allowing the green sunfish and potential prey to see each other, but preventing the green sunfish from directly attacking prey. This set-up represented the treatment portion of the experiment. Observations continued for another 1.25 h. As a final step we removed the clear partition to allow direct interactions between predator and prey.

**Experiment II: Crayfish predation upon native fish**

We had three objectives for the predation experiments, to determine: 1) if crayfish would consume native fish; 2) if native fish used different portions of the water column or used shelter differentially when in the presence of crayfish; and 3) if there were differences in fish mortality due to sex or size of crayfish, and size of fish.

Water level was kept at 22.5 cm throughout the experiment. We placed a clay roof tile in the tank to provide shelter for crayfish. Each replicate took 2 days. On the first day, we ran the control experiment, in which we observed 4 fish of a given size class (e.g., 3-4 cm) in the absence of crayfish. Fish were placed in the tank and allowed to acclimate for at least 15 min, then we added 0.05 g of flake fish food. We initiated 2 h of observations 5 min after food was added. We noted the location of fish in the water column (either upper, middle, or lower sections) at 10-min increments. In addition, for eight 5-min periods we noted if fish used the shelter.

On the second day we noted any fish mortality (i.e., if fish were missing from the tank), then ran the treatment portion of the experiment. Two hours before the trial began, a plexiglass partition was inserted to divide the tank in half, with the fish restricted to one side. The 2-h period allowed animals to adapt to their new surroundings before the treatment began. A crayfish was inspected, sexed and its carapace measured before placing it on the other side of the tank. Only crayfish with a complete pair of chelae and antennae were used. We assumed that the animals could see and sense each other though the plexiglass but could not come into physical contact. After 2 h, the plexiglass partition was removed. Animals were again fed 0.05 g of flake fish food, and we collected data identical to that taken in the control. In addition, for eight 5-min periods we counted the number of times crayfish lunged at fish or captured and ate fish. We also recorded how much time was spent by fish and crayfish inside the single shelter. On the third day we recorded fish mortality.

### Data analysis

For the competition data, we used t-tests to compare the percent of time crayfish and fish used the shelter during the two treatments, the number of displacements from shelter, the number of attacks between prey, and if green sunfish attacked the two prey species differentially. We used Pearson correlations to evaluate if fish size was correlated with number of displacements from shelter, number of green sunfish attacks, or percent of time spent in shelter.

We analyzed the predation data with repeated measures multivariate analysis of variance (MANOVA) to test for differences in the amount of time that fish spent in each section of the water column during the control and treatment periods. We used Wilk's lambda *P*-values to determine significance. We conducted t-tests to compare fish mortality as a function of treatment and crayfish gender or size. For all statistical tests, we used an $\alpha$-level of 0.05 to determine statistical significance.

## RESULTS

### Experiment I: Competition for shelter in presence of predatory fish

In the competition experiments, we refer to native fish and crayfish as "prey" and the green sunfish as the "predator". In the control portion of the competition experiment, a green sunfish was separated from the crayfish and fish by an opaque partition. In the treatment portion, the opaque partition was removed, and the green sunfish and prey could react to each other but sunfish could not consume prey because the clear plastic partition remained in the tank.

#### Gila chub

We ran 9 trials to evaluate competition for shelter between crayfish and Gila chub. Crayfish ranged from 22.6-29.0 mm carapace length (CL). We used 2 green sunfish in Gila chub experiments. Five trials used 3-4 cm Gila chub, and 4 trials used 4-6 cm Gila chub. There was no significant correlation between actual size of Gila chub and percent of time that they spent in shelter, number of

times they were displaced from shelter by crayfish, or number of times that they were attacked by green sunfish (Pearson correlations $<0.4$ ; $P>0.3$).

We noted minor differences between the 2 green sunfish used in this experiment. One green sunfish was more passive than the other, and never attacked the prey through the partition. However, this passivity did not affect fish and crayfish response. There was no difference in shelter use by either prey species, regardless of which green sunfish was used in a given trial (two-sample t-tests; $P>0.4$).

Gila chub never attacked crayfish. Crayfish attacked Gila chub only once when they were outside the shelter. Six other attacks were associated with shelter displacement. The average number of attacks, however, was not significantly different from zero ($\bar{x}=0.78 \pm 0.52$ SE attacks/trial for all treatments: one-sample t-test: $t=1.49$, df=9, $P=0.17$)

Gila chub did not displace crayfish from shelter during any trial. In contrast, crayfish displaced Gila chub from shelter 5 times during the course of 9 trials. Crayfish successfully defended the shelter 4 times, by repelling a Gila chub that was trying to enter. Average displacements by crayfish during the control period was significantly more than zero ($\bar{x} = 0.56 \pm 0.24$ SE displacements/trial: one-sample t-test: $t=2.29$, df=8, $P=0.05$). It is likely that displacement during control is an artifact of timing: the control period always occurred before treatment. Crayfish may be displacing Gila chub most often when they are first interacting, and then over time the fish ceases to seek shelter, thus reducing number of displacements.

There was no significant difference in the amount of time spent in shelter between crayfish and Gila chub when the opaque partition was present (the control period) and they could not interact directly with the green sunfish (Figure 1 and Table 1). However, when the opaque divider was removed and green sunfish and prey could interact through the clear partition, crayfish spent significantly more time in shelter than Gila chub. Green sunfish attempted to attack prey frequently, however there was no preference for prey type when only the clear partition was in place (Table 2). Although Gila chub had used the interstitial space during control and treatment periods, they never sought interstitial cover when green sunfish had direct access to them, following removal of the clear partition. After partition removal, green sunfish always consumed Gila chub before crayfish.

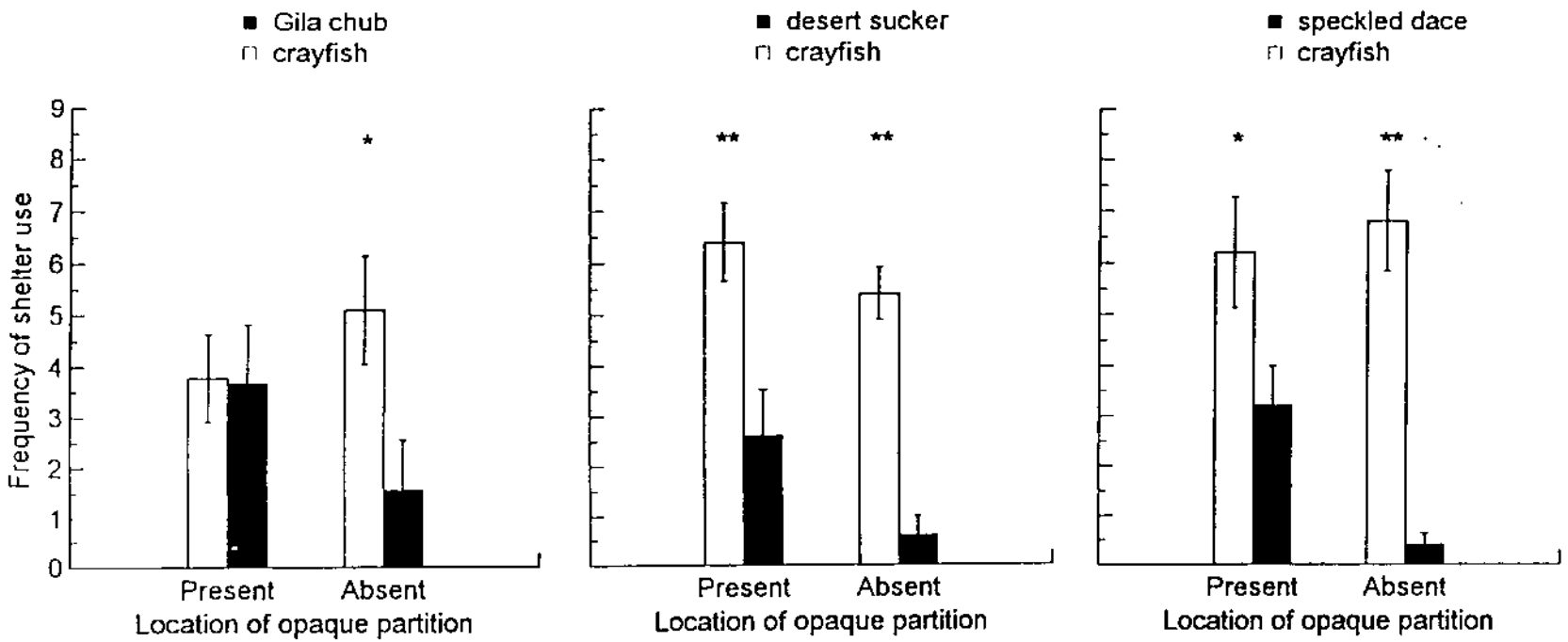


Figure 1. Use of shelter ($\bar{x} \pm SE$) by Gila chub, desert sucker, and speckled dace as compared to crayfish during control and treatment periods. Asterisks indicates significance at $P \leq 0.05$* or $P \leq 0.01$**.

Table 1. T-test analyses on shelter use (number of times animal used shelter during six 5-min observations per treatment period) during competition experiments in 113-L aquaria. The treatment consisted of removing the opaque partition so that green sunfish and prey species could see each other and interact through a clear partition.

| Treatment | Prey species | *t* | df | *P* |
|---|---|---|---|---|
| Control | Gila chub | 0.08 | 16 | 0.940 |
| | crayfish | | | |
| Treatment | Gila chub | 2.45 | 16 | 0.026 |
| | crayfish | | | |
| Control | desert sucker | 3.19 | 8 | 0.013 |
| | crayfish | | | |
| Treatment | desert sucker | 7.41 | 8 | 0.0001 |
| | crayfish | | | |
| Control | speckled dace | 2.249 | 8 | 0.055 |
| | crayfish | | | |
| Treatment | speckled dace | 6.40 | 4.5 * | 0.002 |
| | crayfish | | | |

Table 2. Number of attacks by green sunfish during competition experiments in 113-L aquaria.

| Prey species | Number of attacks ($\bar{x} \pm$ SE) | *t* | df | *P* |
|---|---|---|---|---|
| Gila chub | 0.67 ± 0.29 | 0.66 | 8 | 0.525 |
| crayfish | 1.56 ± 1.20 | | | |
| desert sucker | 5.60 ± 1.36 | -1.68 | 8 | 0.132 |
| crayfish | 2.20 ± 1.50 | | | |
| speckled dace | 6.40 ± 1.36 | -0.08 | 4.7 * | 0.937 |
| crayfish | 6.00 ± 4.56 | | | |

* These df are lower because we used t-tests that account for unequal variances. For all other comparisons we could assume equal variances (Prob > F' > 0.1).

Desert sucker

We ran 5 trials to evaluate competition for shelter between crayfish and desert sucker. Crayfish ranged from 20.5-30.4 mm CL. Desert suckers ranged from 71-84 mm TL. We used four green sunfish in these experiments. We measured no significant correlation between size of desert sucker and percent of time that they spent in shelter, number of times they were displaced from shelter by crayfish, or number of times they were attacked by green sunfish (Pearson correlations $<0.3$ ; $P>0.7$).

Desert suckers used the interstitial cover significantly less than did crayfish during both control and treatment periods (Table 1; Figure 1). Suckers used shelter less during treatment than in control periods, but the difference was marginally significant (paired t-test: $t=1.98$, df=8, $P=0.08$). During 5 trials, crayfish attacked desert suckers 4 times, and displaced them from cover 3 times. Crayfish attacks on desert sucker were not significantly different from zero ($\bar{x}=0.80 \pm 0.37$ SE attacks/trial for all treatments; one-sample t-test: $t=2.14$, df=5, $P=0.10$). One sucker displaced a crayfish out of shelter but was then immediately displaced by the crayfish. When the opaque partition was removed, green sunfish did not show a significant preference for either prey (Table 2). When green sunfish attacked desert suckers through the partition, suckers did not use shelter but darted away and remained motionless on the substrate.

Speckled dace

We ran 5 trials to evaluate competition for shelter between crayfish and speckled dace. Crayfish ranged in size from 20.0-22.9 mm CL, and speckled dace ranged from 42-45 mm TL. We used four green sunfish in these experiments. We did not analyze differences in variables by fish length since speckled dace were so similar in size.

Speckled dace responded to the competition experiments in a manner similar to desert sucker. Speckled dace used the interstitial shelter significantly less than did crayfish during both control and treatment periods (Table 1; Figure 1). Speckled dace used shelter less during treatment periods than during the control ($t=3.35$; df=4.7; $P=0.023$). Crayfish never displaced speckled dace. During 5 trials, crayfish attacked dace 8 times. Green sunfish showed no preference for either prey (Table 2).

**Experiment II: Crayfish predation upon native fish**

Predation on Gila chub

Crayfish used in the predation experiments ranged from 37.6-45.2 mm CL ($\bar{x}$ = 41.3 ± 0.33 SE). All Gila chub were 2-3 cm TL. All crayfish used in trials with Gila chub were male. Out of a total 13 trials, crayfish consumed 1 of 4 Gila chub in 3 trials. There was no mortality in control trials. Average mortality over all trials was 7.1% (± 0.03 SE). We did not consider crayfish size as a factor because there was no variation in mortality (either 0 or 25%). Gila chub used all levels of the water column equally and did not change their use of the water column when in the presence of crayfish (Tables 3 and 4). We saw no predation on Gila chub during our observations; apparently Gila chub were preyed upon at night.

Predation on desert sucker

We evaluated crayfish predation on desert suckers in 6 trials. Crayfish ranged from 44-49.5 mm CL and desert suckers were between 3-4 cm TL. Crayfish consumed desert suckers in 4 of 6 trials; mortality in these cases was 25%, 50%, 50%, and 75%. Average mortality over the entire experiment was 33.3% (± 12.36 SE). We saw no mortality in control trials. Desert suckers did not alter their use of the water column in the presence of crayfish (Tables 3 and 4); suckers spent most of their time in the lower water column, on or near the bottom of the tank. We did not observe any direct predation events between desert sucker and crayfish; predation apparently occurred at night.

## DISCUSSION

In experiments evaluating competition for shelter between crayfish and native fish (Gila chub, desert sucker, and speckled dace), native fish never attacked the crayfish, and only once did a native fish (a 77-mm desert sucker) displace a crayfish from the shelter. Crayfish attacked fish in 7 of 19 trials (9 for Gila chub and 5 each for desert sucker and speckled dace). In all trials the native fish explored the shelter at least once; yet they never used the shelter when threatened by the green sunfish predator. When the opaque divider was removed and the green sunfish could see the prey, no

Table 3. Frequency of water column use by native fish during predation experiments. Maximum possible frequency is 4 fish * 12 observations = 48.

| Fish species | Crayfish treatment | Water column level | Frequency of use ($\bar{x}$ ± SE) | Number of trial experiments |
|---|---|---|---|---|
| Gila chub | Absent (control) | Upper | 17.92 ± 2.61 | 13 |
| | | Middle | 13.00 ± 1.51 | |
| | | Lower | 17.08 ± 3.04 | |
| | Present (treatment) | Upper | 16.92 ± 2.85 | 13 |
| | | Middle | 15.69 ± 2.23 | |
| | | Lower | 15.38 ± 2.95 | |
| desert sucker | Absent (control) | Upper | 6.83 ± 3.59 | 6 |
| | | Middle | 3.50 ± 1.78 | |
| | | Lower | 37.67 ± 4.86 | |
| | Present (treatment) | Upper | 2.83 ± 0.79 | 6 |
| | | Middle | 4.17 ± 2.01 | |
| | | Lower | 41.00 ± 2.54 | |

Table 4. MANOVA results of water column use by native fish during predation experiments. Treatment effect refers to presence of a crayfish predator. A nonsignificant *P*-value for the water column effect indicates that during the control period, the fish used all portions of the water column equally. A nonsignificant *P*-value for the water column by treatment effect indicates that addition of a crayfish predator did not significantly change use of the water column by the given fish species.

| Fish species | Null hypotheses | Wilk's Lambda | F | df (num,den) | *P* |
|---|---|---|---|---|---|
| Gila chub | $H_0$: No water column effect | 0.932 | 0.842 | 2,23 | 0.444 |
| | $H_1$: No water column* treatment effect | 0.960 | 0.481 | 2,23 | 0.624 |
| desert sucker | $H_2$: No water column effect | 0.095 | 43.10 | 2,9 | 0.0001 |
| | $H_3$: No water column* treatment effect | 0.811 | 1.049 | 2,9 | 0.389 |

preference for prey type was evident. However, when all dividers were raised and green sunfish had access to both prey species, green sunfish always consumed the native fish before crayfish.

Our results provide evidence that these three native fish species may be inferior competitors with crayfish for shelter when shelter is a limited resource. The implication is that these native fishes become more vulnerable to predation when crayfish are present.

In predation experiments, crayfish preyed upon two native fish species at different rates. Desert suckers suffered higher mortality than Gila chub when in tanks with crayfish. Desert suckers may experience higher mortality because of their preference for benthic areas, where crayfish are more likely to encounter them. In addition, desert suckers were slower to respond to active crayfish than were Gila chub. Although we saw no fish captures in our lab experiments, in previous lab work with 2-3 cm *Catostomus* spp. (*C. insignis* or *C. clarki*), we frequently saw crayfish capture and consume suckers (Carpenter and McIvor 1998). These data provide evidence that crayfish will prey on two native fish species if they become vulnerable. Given the propensity for desert sucker and Gila chub to remain inactive or resting on the substrate, coupled with the nocturnal foraging habits of crayfish, it is reasonable to expect that similar encounters occur in field situations.

Predation by non-native piscivores on juvenile fish may be a factor in the decline of native Arizona fishes (Ruppert et al. 1993). Minckley (1983) proposes that before the introduction of non-native fish, shallow shoreline areas were free of piscivorous fish. Thus it may be that selection of shallow shoreline areas as cover is the primary predator avoidance behavior of juvenile native fish in Arizona streams, as it is in many other aquatic systems (Power 1987, McIvor and Odum 1988, Ruiz et al. 1993). Crayfish can readily access these shallow areas, thereby rendering shallow areas less suitable refuge for juvenile native fish. We found that Gila chub sought cover infrequently, even when being actively pursued by green sunfish. It is possible that these small chub did not seek cover because they were not familiar with the interstitial cover types provided in our experiments. In Sabino Creek, YOY Gila chub (2-3 cm) are usually found in shallow water areas (< 20 cm in depth) that are dominated by substrate sizes of < 16 mm; therefore these areas usually lack interstitial cover, in contrast to deeper stream sections containing boulders and cobble (Dudley 1995).

We observed neither desert sucker nor Gila chub altering their use of the water column when in the presence of a crayfish predator; this suggests a lack of behavioral plasticity. Such seeming lack of recognition of a predator and the danger it represents is likely due to the lack of a common evolutionary history with crayfish. If native fish show inappropriate behavior because they did not

evolve in the presence of a benthic nocturnal predator, then the long-term outlook for population viability of these two species of native fishes where they co-occur with crayfish is not promising.

Some may argue that predation rates observed in laboratory studies are artifacts of the confining nature of the aquaria. Admittedly, the aquarium setting is less extensive and less structurally complex than natural stream conditions. Nonetheless, the density of Gila chub and crayfish was similar to (or even less than) that found in pools in Sabino Canyon during mid-summer drought (see Part I of this report).

In summary, we determined that in a laboratory setting in small (113-L) aquaria, three species of native fishes were inferior competitors to crayfish for shelter. Additionally, when in close proximity, the crayfish becomes the predator, the native fishes the prey. These data point out the vulnerability of small-sized native fishes of three species where they occupy the same stream habitats as exotic crayfish.

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